State of California

Memorandum

To : Barry Cortez, Chief
Pesticide Registration Branch

Date : December 3, 1993

Place : Sacramento

Phone: 4-1285

From : **Department of Pesticide Regulation** - Larry Nelson, Chief
Medical Toxicology Branch

Subject : Hydrogen Cyanamid Risk Characterization Document

A risk characterization document for the pesticide active ingredient hydrogen cyanamid is forwarded for distribution in accordance with Pest Management Division policy #87-3. Please also provide copies to Both USEPA OPP Special Review and Registration Division and the USEPA OPP Health Effects Division.

SURNAME			

HYDROGEN CYANAMIDE

RISK CHARACTERIZATION DOCUMENT

Medical Toxicology and Worker Health and Safety Branches
Department of Pesticide Regulation
California Environmental Protection Agency

December 1, 1993

EXECUTIVE SUMMARY

INTRODUCTION

Hydrogen cyanamide is an active ingredient which was patented by SKW Trostberg Aktiengesellschaft as a plant growth regulator. This document concerns a new Section 3 registration for hydrogen cyanamide (Trade name: Dormex®) which can be sprayed on dormant grape vines. Hydrogen cyanamide causes chemical vernalization and uniform bud break. It may be applied in a coarse large droplet spray with a 4% (v/v) dilution of Dormex® and 1/4 - 1/2% non-ionic surfactant on pruned grapevines vines between December 1 and January 31.

THE RISK ASSESSMENT PROCESS

Hydrogen cyanamide entered the risk assessment process because of possible adverse effects identified in sub-chronic toxicity, chronic toxicity, and oncogenicity studies. The risk assessment process consists of four aspects: hazard identification, dose response assessment, exposure evaluation, and risk characterization.

Hazard identification entails review and evaluation of the toxicological properties of each pesticide. The dose-response assessment then considers the toxicological properties and estimates the amount which could potentially cause an adverse effect. The amount which will not result in an observable or measurable non-oncogenic effect is called the No-Observed-Effect Level, NOEL. In general, it is assumed that oncogenic effects of a chemical may occur at all dosages. The ability of a chemical to cause tumors is indicated by its potency.

A basic premise of toxicology is that at a high enough dose, virtually all substances will cause some toxic manifestation. Chemicals are often referred to as "dangerous" or "safe", as though these concepts were absolutes. In reality, these terms describe chemicals which require low or high dosages, respectively, to cause toxic effects. Toxicological activity is determined in a battery of experimental studies which define the kinds of toxic effects which can be caused, and the exposure levels (doses) at which effects may be seen. State and federal testing requirements mandate that substances be tested at doses high enough to produce toxic effects, even if such testing involves chemical levels many times higher than those to which people might be exposed.

In addition to the intrinsic toxicological activity of the pesticide, the other parameters critical to determining risk are the exposure level, frequency and duration. The purpose of the exposure evaluation is to determine the potential exposure pathways and the amount of pesticide likely to be delivered through those routes.

The risk characterization then integrates the observed toxic effects (from laboratory studies conducted with high dosages of pesticide) with potential human exposures at low dosages. The likelihood of potential, non-oncogenic adverse health effects in people is generally expressed as a margin of safety. The margin of safety is a ratio of the dosage which produced no effects in laboratory studies to the dosage humans might potentially receive. For oncogenic effects, an additional lifetime risk of cancer may be calculated by multiplying the cancer potency of the pesticide times the estimated exposure dosage.

TOXICOLOGY

Based on the currently available toxicity information, DPR concluded that hydrogen cyanamide causes adverse effects in the liver, thyroid, kidney, ovaries, and testes of laboratory animals. DPR has further concluded that, in the absence of additional data to the contrary, hydrogen cyanamide has the potential to cause similar effects in humans.

EXPOSURE ANALYSIS

Exposures of mixer/loaders, applicators, and supervisors were determined under actual application conditions. Estimates of the amount of hydrogen cyanamide absorbed by workers in each job category were based on monitoring of cyanamide metabolites in their urine.

CONCLUSIONS

Using laboratory animal toxicity data and worker exposure data, the calculated margins of safety (MOSs) for potential acute exposure of mixer/loaders, applicators and supervisors are considered adequate. MOSs for potential seasonal and chronic occupational exposure to hydrogen cyanamide are also considered adequate when the mitigation measures on the label are followed. Additional lifetime risks of cancer from potential lifetime exposures to hydrogen cyanamide are considered minimal, and therefore acceptable. MOSs for potential acute exposure of bystanders to drift are considered adequate also.

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DPR acknowledges the review of this document by the
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I. SUMMARY

Hydrogen cyanamide is an active ingredient which was patented by SKW Trostberg Aktiengesellschaft as a plant growth regulator. This document concerns a new Section 3 registration for hydrogen cyanamide (Trade name: Dormex®) which can be sprayed on dormant grape vines. Hydrogen cyanamide causes chemical vernalization and uniform bud break. It may be applied as a coarse large droplet spray with a 4% (v/v) dilution of Dormex® and 1/4 - 1/2% non-ionic surfactant on pruned grapevines vines between December 1 and January 31.

Environmental fate. The photolytic half-life of hydrogen cyanamide in aqueous solution was 29 days at pH5 and 39 days at pH7. Under aerobic conditions, the half-life of cyanamide in sandy loam soil was approximately 1/2 day. Under anaerobic conditions, the half-life in soil was 35 days. Hydrogen cyanamide was only slightly mobile in soil, moving slower in soils with higher organic content. Under field use conditions, the half-life of hydrogen cyanamide varied between 10 and 15 days. No detectable residues were found in grapes from vines which were treated with hydrogen cyanamide. The data strongly suggest that hydrogen cyanamide is rapidly broken down in the environment and is unlikely to persist as an environmental contaminant.

Pharmacokinetics. Oral absorption of hydrogen cyanamide by laboratory animals was 100%. The average absorbed dermal doses in 24 hours by humans and rats were 5.5% and 11.1%, respectively. Approximately 40% of a human oral dose was recovered in the urine in the first 24 hours. For rats, 43% of the oral dose was recovered in the urine. Approximately 95% of the absorbed oral dose in rats or humans was excreted in the first 24 hours. The remainder of dermal or oral doses given to rats or humans was excreted in the feces or exhaled as CO_2. Hydrogen cyanamide did not concentrate in any tissues in the rat. There was no indication of hydrogen cyanamide being converted to cyanide *in vivo* in rats or humans. The principal metabolite excreted in the urine of laboratory animals and humans was *N*-acetylcyanamide.

Acute Toxicity. The oral LD_{50} in rats was 125 mg/kg for technical grade material, and the dermal LD_{50} in rabbits ranged from 742 mg/kg to 901 mg/kg. The inhalation LC_{50} was greater than 1000 mg/m^3. Hydrogen cyanamide caused mild skin (Category IV) and eye (Category III) irritation in the rabbit.

Subchronic Toxicity. Hydrogen cyanamide by the oral route in rats caused hepatotoxicity (hydropic liver cell degeneration, individual liver cell degeneration, enlarged periportal hepatocytes with clumped cytoplasm, and bile duct proliferation), and thyroid toxicity (small follicular lumens without colloid, separated by proliferating epithelial cells and interfollicular cells). The lowest-observed-effect-level (LOEL) for hepatotoxicity (28 days) in rats was 4.6 mg/kg-day. The 28-day no-observed-effect-level (NOEL) for bile duct hyperplasia in rats dosed by gavage was 5 mg/kg-day. In the same 28-day gavage study, the LOEL for thyroid toxicity was 5 mg/kg-day. The 90 day NOEL for thyroid toxicity in rats exposed to hydrogen cyanamide in the diet was 0.8 mg/kg-day. Dogs also exhibited thyroid toxicity in response to hydrogen cyanamide by gavage. The NOEL (90 days) for thyroid toxicity (reduced plasma thyroxin levels) in dogs dosed with hydrogen cyanamide by gavage was 2 mg/kg-day. In the same study, the LOEL for testicular atrophy and oligospermia in the dog, was 0.6 mg/kg-day.

Chronic Toxicity. Hydrogen cyanamide was not oncogenic in the rat, but chronic exposure caused thyroid toxicity (reduced colloid and the formation of micro-follicles, reduced T_3 and thyroxine levels in the plasma). The NOEL for thyroid toxicity in the rat was 1 mg/kg-day. Hydrogen cyanamide was oncogenic in the mouse, causing a significant, dose-related increase in granulosa-theca tumors in the ovary. In addition, hydrogen cyanamide caused nephrotoxicity (fibrosis and scarring, atrophic/basophilic tubules, and vacuolar degeneration and necrosis), chronic cystitis of the urinary bladder, hepatotoxicity (biliary proliferation and centrilobular hypertrophy) in the mouse. The NOEL for mouse hepatotoxicity was 29.5 mg/kg-day. The NOEL for mouse nephrotoxicity and chronic cystitis was 13.7 mg/kg-day. In dogs, hydrogen cyanamide caused thyroid toxicity (lower thyroxine levels), changed clinical chemistry indicating reduced over-all metabolism, testicular effects (neutrophil infiltration of testes, oligospermia), and clinical signs (tremors and excessive salivation). The NOEL for these effects in dogs was 0.2 mg/kg-day.

Genotoxicity. Hydrogen cyanamide was not mutagenic in the Ames test, and did not stimulate unscheduled DNA synthesis *in vitro*. It did induce chromosomal aberrations in Chinese hamster cells *in vitro*. However, it did not produce micronucleus formation *in vivo*. Thus, the genotoxic potential of hydrogen cyanamide is considered equivocal.

Reproductive Toxicity. Hydrogen cyanamide by gavage was not reported to cause any significant histomorphic changes in parental rats or offspring associated with the treatments. No reproductive effects of hydrogen cyanamide were reported in a study acceptable under FIFRA. The adult NOEL was 1.25 mg/kg-day for decrement in body weight. There was no NOEL for neonatal pup survival (days 0-4). The LOEL was 1.25 mg/kg-day. In an earlier, unacceptable study, dietary exposure to hydrogen cyanamide was reported to cause atrophic seminiferous tubules and interstitial cell proliferation in rats.

Developmental Toxicity. In rabbits, the NOEL for maternal toxicity (significant decrement in weight gain) was 6 mg/kg-day. The NOEL for developmental toxicity (retinal folds) was 2 mg/kg-day. Hydrogen cyanamide by gavage caused an increased incidence of diaphragmatic hernias and depression of fetal body weights in rats. The NOEL for developmental effects in rats was 15 mg/kg-day. The maternal NOEL for clinical signs (hypoactivity, hunched posture, fecal and urine stains, protruding eyes, malocclusion, and chromodacryorrhea) in rats was 5 mg/kg-day.

Hazard Identification. The most sensitive toxicological endpoint, a NOEL of 5.0 mg/kg for clinical signs in the rat, was used as the basis for assessing the margins of safety (MOSs) for potential acute occupational exposure.

An Estimated-No-Effect-Level (ENEL) of 0.42 mg/kg-day for neonatal survival in rats, was used for the estimation of safety margins for potential seasonal exposure to cyanamide.

The NOEL (0.2 mg/kg-day) for thyroid toxicity (lower thyroxine levels), changed clinical chemistry indicating reduced over-all metabolism, testicular effects (neutrophil infiltration of testes, oligospermia), and clinical signs (tremors and excessive salivation) in dogs was used for the calculation of safety margins for potential chronic exposure to hydrogen cyanamide.

To assess the additional lifetime risk of cancer from potential lifetime exposures to hydrogen cyanamide, the maximum likelihood estimate (MLE) for human cancer potency of 0.007 $(mg/kg\text{-}day)^{-1}$, with an upper bound (95% confidence level) of 0.02 $(mg/kg\text{-}day)^{-1}$ were used.

Occupational Exposure. The average absorbed daily dosage for mixer/loaders was 11.6 $\pm$ 4.7 *u*g/kg-day, for applicators 5.3 $\pm$ 3.8 *u*g/kg-day, and for supervisors 2.6 $\pm$ 0.5 *u*g/kg-day. The 60-day mean seasonal absorbed daily dosage ranged from to 1.3 *u*g/kg-day for supervisors to 3.9 *u*g/kg-day for mixer/loader/applicators. The mean annual absorbed daily dosage ranged from 0.2 *u*g/kg-day for supervisors to 0.7 *u*g/kg-day for mixer/loader/applicators. The estimates for mean lifetime absorbed daily dosage ranged from 0.1 *u*g/kg-day for supervisors to 0.4 *u*g/kg-day for mixer/loader/applicators.

The estimated absorbed daily dosage of a person working in a nearby field, exposed for 1 hour a day to drift, was 7.5 *u*g/kg-day at 100 feet, 4.1 *u*g/kg-day at 200 feet, and 2.9 *u*g/kg-day at 300 feet.

Risk Characterization. MOSs for the mean potential acute occupational exposures, based on the NOEL of 5 mg/kg-day for clinical signs, ranged from 431 (mixer/loaders) to 1,923 (supervisors). If the 95 percent confidence limit of short-term exposure (mean plus two standard deviations) were considered for each of the job categories, the MOSs would range from 238 (mixer/loaders) to 1,389 (supervisors). MOSs for potential drift exposure of laborers working nearby ranged from 667 at 100 feet to 1,724 at 300 feet. The MOSs for mean potential seasonal exposure, based on an ENEL of 0.42 mg/kg-day for neonatal survival in rats, ranged from 108 (mixer/loader/applicators) to 323 (supervisors). MOSs for mean potential annual occupational exposures, based on a NOEL of 0.2 mg/kg-day for testicular atrophy, thyroid toxicity and clinical signs in dogs, ranged from 286 (mixer/loader/applicators) to 1,000 (supervisors). The maximum likelihood estimates for additional lifetime risks of cancer ranged from 1×10^{-6} (supervisors, mixer/loaders, applicators) to 3×10^{-6} (mixer/loader/applicators), based on ovarian granulosa-theca tumors

observed in the mouse. The 95% upper confidence limit on the maximum likelihood estimates for additional lifetime risks of cancer ranged from 2 x 10^{-6} (supervisors) to 8 x 10^{-6} (mixer/loader/applicators).

Conclusions. Using current toxicity data and estimates from worker monitoring information on hydrogen cyanamide, the calculated margins of safety (MOSs) for potential acute, seasonal, and annual exposures were considered adequate. The maximum likelihood estimate for additional lifetime risk of cancer ranged from 1 x 10^{-6} to 4 x 10^{-6} and were considered acceptable because of the overestimation of lifetime occupational exposure, and the weak weight of evidence supporting the possible occurrence of carcinogenicity. The 95% upper confidence limit on the maximum likelihood estimates for additional lifetime risk of cancer ranged from 2 x 10^{-6} to 8 x 10^{-6} and were also considered acceptable for the same reasons.

Likewise, MOSs for potential acute exposure of bystanders to drift, at distances from 100 to 300 feet from the site of operations, were adequate.

II. INTRODUCTION

A. CHEMICAL IDENTIFICATION

Hydrogen cyanamide (Trade name Dormex®) is a plant growth regulator which is used to promote uniform budbreak of grape vines. Hydrogen cyanamide entered into the risk assessment process because of possible adverse effects identified in subchronic, chronic toxicity, and oncogenicity studies in laboratory animals. Under this Section 3 registration of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Dormex® formulation is a liquid concentrate containing 51.0% active ingredient. The formulation is applied to pruned grapevines as a coarse large droplet spray of a 4% (v/v) solution containing 1/4 - 1/2% non-ionic surfactant. Approximately 50 - 100 gallons of spray is required per acre of grapevines. A single application each year, between December 1 and January 31, is recommended.

B. REGULATORY HISTORY

The time-weighted-average (TWA) threshold limit value (TLV) for hydrogen cyanamide in the workplace is 2 mg/m^3 (ACGIH, 1986). The Occupational Safety and Health Administration lists the permissible exposure limit (PEL) for hydrogen cyanamide as 2 mg/m^3. The National Institute for Occupational Safety and Health (NIOSH) has adopted this same value, 2 mg/m^3, as the recommended exposure limit (REL) (NIOSH, 1992). Emergency use registrations (Section 18 of FIFRA) were granted for hydrogen cyanamide from 1987 to 1992 in California.

C. TECHNICAL AND PRODUCT FORMULATIONS

Hydrogen cyanamide comes in the form of a liquid concentrate (Dormex®) containing 51% active ingredient and 49% inert ingredients.

D. USAGE

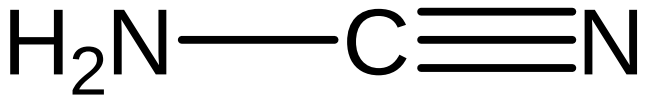
Between 1987 and 1990, an annual average of 205,352 lbs of hydrogen cyanamide was used under the emergency use registrations to promote chemical vernalization and uniform bud break in grapevines in the Imperial and Coachella Valleys in California.

E. ILLNESS REPORTS

No illnesses associated with the agricultural use of hydrogen cyanamide have been reported to DPR. Several published reports indicate that cyanamide, used in aversion therapy for alcoholics, caused the formation of "ground glass" inclusion bodies in hepatocytes, and hepatitis (Vazquez and Cervera, 1980; Thomsen and Reinicke, 1981; Vazquez *et al.*, 1983a,b; Villegas, 1984; Bruguera *et al.*, 1986).

F. PHYSICAL AND CHEMICAL PROPERTIES[a]

Chemical Name:	hydrogen cyanamide
CAS#	420-04-2
Common Name:	hydrogen cyanamide
Empirical Formula:	CH_2N_2
Chemical Structure:	



Molecular Weight:	42.04 g/mol
Melting Point:	45-46°C
Vapor Pressure:	5×10^{-3} Pa @20°C
Solubility (water):	100 g/100 ml @ 43°C
(organic solvents)	soluble in alcohols
Octanol/Water Partition Coefficient	-0.82 @ 20°C

a/ Merck Index

G. ENVIRONMENTAL FATE

Summary- The photolytic half-life of hydrogen cyanamide in aqueous solution was 29 days at pH5 and 39 days at pH7. Under aerobic conditions, the half-life of cyanamide in sandy loam soil was approximately 1/2 day. Under anaerobic conditions, the half-life in soil was 35 days. Hydrogen cyanamide was only slightly mobile in soil, moving slower in soils with higher organic content. Under field use conditions, the half-life of hydrogen cyanamide varied between 10 and 15 days. No detectable residues were found in grapes from vines which were treated with hydrogen cyanamide. The data strongly suggests that hydrogen cyanamide is rapidly broken down in the environment and is unlikely to persist as an environmental contaminant.

Photodegradation

An aqueous photolysis study was conducted with 20 *u*g/ml ^{14}C-cyanamide (23.7 mCi/mmol) at 25°C in solutions buffered at pH7 and pH5 under an Atlas 6500 watt xenon arc lamp for 30 days (Schmidt,

1991a). ^{14}C-cyanamide degraded in aqueous solution with half-lives of 29 and 39 days at pH 5 and 7 respectively. The half-lives in the dark were calculated as greater than 100 days for both systems.

Microbial Degradation

Metabolism of ^{14}C-cyanamide under aerobic conditions in the dark was investigated in sandy loam soil treated with 33 *u*g cyanamide/g soil at a temperature of 25°C for 14 days (Schmidt, 1991b). Mineralization of ^{14}C-cyanamide to $^{14}CO_2$ proceeded rapidly under aerobic conditions, with 20% of the cyanamide converted to CO_2 in 12 hours, and greater than 90% converted in 5 days. The half-life of cyanamide under aerobic conditions was estimated to be approximately 1/2 day (Schmidt, 1990a).

Anaerobic soil metabolism of ^{14}C-cyanamide was investigated under dark conditions at 25°C for sixty days on sandy loam soil dosed at 33 ug cyanamide/g soil (Schmidt, 1991c). The half-life of cyanamide under anaerobic conditions was 35 days, calculated using first order kinetics (Schmidt, 1990b).

Mobility

Three different water saturated sandy soils (89.7 % sand, 9.1% silt, 1.2% clay; 82.7% sand, 10.3% silt, 7% clay; 68.1% sand, 26.9% silt, 5% clay) were packed into 35 cm x 5 cm glass columns and 3.92 mg cyanamide was applied to the top of each column (Rombke, 1990). After two days of continuous elution with 393 ml of water, 2.8%, 1%, and 0.13% of the applied material had been eluted from the respective soil columns. This indicated that hydrogen cyanamide was only slightly mobile in soil.

Field Dissipation

Hydrogen cyanamide was applied to dormant grape vines at the maximum approved application rate (17.3 lbs active ingredient/acre) during December (Siemer, 1991). Hydrogen cyanamide in the wet areas around the drip emitters had a half-life of 15 days, while the dry soil samples indicated a half-life of 10 days.

Plant Residues/Metabolism

Hydrogen cyanamide (10% formulated material by volume in 100 gallons of water) was applied to dormant grape vines in the Fresno area. Analyses of both immature and mature Red Flame grapes (105 days post treatment) revealed no detectable residues (detection limit 0.01 ppm) (Oswald, 1991).

III. TOXICOLOGY PROFILE

A. PHARMACOKINETICS

Summary. Oral absorption of hydrogen cyanamide by laboratory animals was 100%. The average absorbed dermal doses in 24 hours by humans and rats were 5.5% and 11.1%, respectively. Approximately 40% of a human oral dose was recovered in the urine in the first 24 hours. For rats, 43% of the oral dose was recovered in the urine. Approximately 95% of the absorbed oral dose in rats or humans was excreted in the first 24 hours. The remainder of dermal or oral doses given to rats or humans was excreted in the feces or exhaled as CO_2. Hydrogen cyanamide did not concentrate in any tissues in the rat. There was no indication of hydrogen cyanamide being converted to cyanide *in vivo* in rats or humans. The principal metabolite excreted in the urine of laboratory animals and humans was *N*-acetylcyanamide.

Oral and intravenous - rat

HSD:SD rats (5/sex/group) were given [^{14}C]-hydrogen cyanamide (99.1% purity; specific activity 14.8 mCi/mmol) intravenously (1 mg/kg); by gavage (1 mg/kg) in a single dose; by gavage (1 mg/kg) in a single dose following 14 daily oral doses of 1 mg/kg of non-labeled material; by gavage (20 mg/kg) as a single oral dose (Struble, 1992). Regardless of dosing regime or route, the radiolabel did not concentrate in any tissues. Following i.v. dosing, radiolabel was excreted as ^{14}CO2 (10.5% in males, 5.5% in females), in the feces (15% in males, 13% in females), and in the urine (69% in males, 78% in females). Following oral dosing with 1 mg/kg, the mean ^{14}C recovery in expired CO_2 ranged from 7.1% to 15% for males, and 3.8% to 5.8% for females. The corresponding values for expired $^{14}CO_2$ after the 20 mg/kg oral dose were 2.3% for males and 1.5% for females. The mean ^{14}C recovery in urine was 66.9% to 82.6% in males and 77.6% to 90.7% in females dosed orally with 1 mg/kg. After the 20 mg/kg oral dose, 95% of the ^{14}C was excreted in the urine for both males and females. After oral dosing (regardless of amount), fecal excretion accounted for 2.8% to 4.2% of the dose in males and 2.9% to 4.1% in females. Within the first 24 hours, males excreted 77.3% to 92%, and females excreted 85% to 91.2% of an oral dose. The total percentage of administered radiolabel excreted by male and female rats following oral or intravenous dosing were not significantly different. This suggests that oral absorption was 100%.

Oral and Intravenous - rat and dog

Sprague-Dawley rats (4 animals/time point) were dosed by gavage with 2 mg/kg hydrogen cyanamide (purity unstated), and plasma levels were monitored at 1, 5, 7, 10, 20, 30, 45, 60, 90, and 120 minutes after dosing (Obach *et al.*, 1989). In the second part, 2 mg/kg hydrogen cyanamide was administered intravenously to rats, and blood samples were taken by cardiac puncture at 1, 3, 10, 15, 20, 45, 60, 90, and 120 minutes following administration. In the rat, after i.v. or oral administration, cyanamide had a half life of 30 minutes; a total plasma clearance of 117 ml/kg-minute; and a mean residence time of 26 minutes. The absolute oral bioavailability was approximately 69%.

Dogs in the study received 4 mg/kg hydrogen cyanamide orally (gavage) or 1, 2, and 4 mg/kg via the intravenous route. The time course of plasma concentrations was determined up to 360 minutes with each regime. Statistically significant changes were observed in plasma clearance values (12.6 to 19.7 ml/kg-min), half life values (39 to 61 minutes), and mean residence times (50 to 79 minutes) with the various doses and routes of exposure. Plasma clearance decreased when the dose increased ($P<0.01$). The peak plasma concentrations after oral administration were achieved at 30 minutes, and the absolute oral bioavailability was approximately 65%.

Although bioavailability in rats (69%) and dogs (65%) was substantially less than 100%, all other metabolic studies indicated complete absorption (Deitrich *et al.*, 1976; Shirota *et al.*, 1984; Obach *et al.*, 1986; Struble, 1992). Because the bioavailability was estimated from chromatographically measured, unlabeled cyanamide in the blood, the possibility of a first-pass effect (metabolism of cyanamide by the liver before compound reached the general circulation) can not be discounted. The data were considered supplemental.

Oral and Intravenous - dog

Three male beagle dogs (9-11 kg) were dosed with [^{14}C]-cyanamide (0.04 mmol/kg, 12.0 and 15.1 *u*Ci) orally, or an intravenous dose (0.04 mmol/kg, 13.9 *u*Ci) (Shirota *et al.*, 1984). Approximately 0.3% of the oral, or the intravenous dose was excreted in the feces. Urinary excretion accounted for most of the radiolabel administered through the intravenous (83.6%) or oral routes (87%). Following oral dosing, *N*-acetylcyanamide constituted the major portion (87%) of the urinary radioactivity excreted in the first 27 hours. Unchanged hydrogen cyanamide was present in the urine as 11% of the administered oral dose. The study was considered supplemental.

Dermal - rat

Male Crl:CD(SD)BR rats (24 per dose; 4 per time point) were dermally dosed with 0.1, 1.0, or 10 mg [^{14}C]-hydrogen cyanamide (49% w/w; 15 mCi/mmol) for 0.5, 1, 2, 4, 10, or 24 hours (LeVan, 1989). The total amount of systemically absorbed hydrogen cyanamide, calculated by adding the amount of radioactivity recovered in the excreta and carcass, averaged 1.8%, 2.8%, and 11% of the dose at 24 hours for doses of 0.1, 1.0, and 10 mg per animal, respectively. The study was considered supplemental.

Intraperitoneal - rat

Sprague-Dawley rats (150-250 g; number unstated) of both sexes were injected with 10 *u*Ci [^{14}C]-hydrogen cyanamide/kg intraperitoneally (Deitrich *et al.*, 1976). Within 6 hours, 94% of the applied dose was excreted in the urine of the animals. Negligible amounts (1.4% of the applied dose) were found in the expired CO_2 in the first 6 hours. The ED_{50} (effective dose for 50% of the animals to respond) for inhibition of aldehyde dehydrogenase in the liver was 3 mg/kg. The study was considered supplemental.

Oral and Dermal - human and rat

Six male human volunteers (aged 33-67 yrs) took 20 mg of cyanamide orally and urine samples were collected every 12 hours for 48 hours (Gloxhuber *et al.*, 1989). Approximately 40% $\pm$ 11% of the oral dose was recovered in the urine in the first 24 hours. This represented 97% of the total cyanamide recovered in the urine. For dermal absorption, 10 mg of cyanamide was applied in a gauze patch under a watertight rubber foil to each forearm of the 6 individuals for a total of 6 hours. After removal of the patches, the contaminated skin areas were rinsed with water and wiped dry. Urine was collected every 12 hours for 48 hours after the dermal application. Approximately 2.2% $\pm$ 1.1% of the dermally applied dose was excreted in the urine. The average absorbed dermal dose was equal to percentage of the dose measured in the urine (2.2%) divided by the fraction of the absorbed dose found in the urine from oral studies (0.4), or 5.5%. Even though *in vitro* findings indicated hydrogen cyanamide could be converted to cyanide (Shirota *et al.*, 1987a,b; DeMaster *et al.*, 1988), there was no indication of this occurring *in vivo* in humans.

In the second part of the study, 4 male Wistar rats were given dosages of 10 mg/kg by gavage. An average of 42.7 $\pm$ 5.8 % of the oral dose was excreted in the urine within the first 24 hours. This constituted 94% of the total cyanamide recovered in the urine. The results of this study were also published in summary form in the open literature (Mertschenk *et al.*, 1991).

B. ACUTE TOXICITY

The acute toxicity of hydrogen cyanamide is summarized in Table 1. A lethal dose of hydrogen cyanamide in humans was reported to be 40-50 g (Yegiazarov, 1971). Hydrogen cyanamide caused mild skin (Category IV) and eye irritation (Category III) in the rabbit (Liggett, 1989; van Beek, 1974). However, hydrogen cyanamide caused strong dermal sensitization in guinea pigs (Til and Veldhuysen, 1982).

Table 1 - Acute toxicity of hydrogen cyanamide in laboratory animals

Species	Sex	Results	Reference
		TECHNICAL	
Oral LD_{50}			
Rat	M/F	150 mg/kg	1
Dermal LD_{50}			
Rabbit		undetermined	2
Rabbit	M	901 mg/kg	3
Rabbit	F	742 mg/kg	3
Inhalation LC_{50}			
Rat	M/F	> 1000 mg/m^3	4

References- 1. Engel, 1973a; 2. Engel, 1973b; 3. Hazleton, 1988; 4. Engel, 1973c.

C. SUBCHRONIC TOXICITY

Summary. Hydrogen cyanamide by the oral route in rats caused hepatotoxicity (hydropic liver cell degeneration, individual liver cell degeneration, enlarged periportal hepatocytes with clumped cytoplasm, and bile duct proliferation), and thyroid toxicity (small follicular lumens without colloid, separated by proliferating epithelial cells and interfollicular cells). The lowest-observed-effect-level (LOEL) for hepatotoxicity (28 days) in rats was 4.6 mg/kg-day. The 28-day no-observed-effect-level (NOEL) for bile duct hyperplasia in rats dosed by gavage was 5 mg/kg-day. In the same 28-day gavage study, the LOEL for thyroid toxicity was 5 mg/kg-day. The 90 day NOEL for thyroid toxicity in rats exposed to hydrogen cyanamide in the diet was 0.8 mg/kg-day. Dogs also exhibited thyroid toxicity in response to hydrogen cyanamide by gavage. The NOEL (90 days) for thyroid toxicity (reduced plasma thyroxin levels) in the dog dosed with hydrogen cyanamide by gavage was 2 mg/kg-day. In the same study, the LOEL for testicular atrophy and oligospermia in the dog, was 0.6 mg/kg-day.

Dietary - Rat

Wistar rats (10/sex/dose) were fed a diet containing Cyanamide L 500 (50% w/w active ingredient) at 0, 20, 60 or 180 ppm for 90 days (Til *et al.*, 1975). No treatment-related effects on body weight, organ weights, or histopathology of the liver were reported. Histopathological effects on the thyroid were observed in three males and two females at 180 ppm and in one male at 60 ppm. The effects consisted of predominantly small follicular lumens without colloid, separated by proliferating epithelial cells and interfollicular cells. The NOEL for thyroid toxicity was 20 ppm (approximately 0.8 mg cyanamide/kg-day from food consumption data). The study was unacceptable under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) guidelines series 82-1 because the histopathological examinations of designated tissues were not performed, lack of clinical chemistry and hematology, and the data were in summary form (USEPA, 1984). The data were considered supplemental.

Wistar rats (10/sex/dose) were fed a diet containing Cyanamide L 500 (50% w/w active ingredient) at 0, 100, 300, 1000 or 3000 ppm for 28 days (Til *et al.*, 1974). Significant ($P<0.05$) reduction in hemoglobin values in both males (92% of control) and females (94% and 90% of control) were seen at 1000 and 3000 ppm, respectively. Significant ($P<0.05$) increases in relative liver weight (males- 123% and 190%; females- 133% and 221% at 1000 and 3000 ppm, respectively) and relative kidney weight (males- 154%; females-

141% at 3000 ppm) were observed. The significant (P<0.05) decrement in body weight gain in both males and females correlated with a decrement in food consumption. Distinct histopathological changes in the livers of males and females of all treatment groups were observed. The changes varied from hydropic liver cell degeneration accompanied by a very marked bile duct proliferation in the 3000 ppm and 1000 ppm groups, to the occurrence of varying numbers of enlarged periportal hepatocytes showing clumped cytoplasm and individual liver cell necrosis in the 300 ppm and 100 ppm groups. There was no NOEL for hepatotoxicity. The LOEL for hepatotoxicity was 100 ppm (an average of 4.6 mg cyanamide/kg-day based on consumption data). The study was unacceptable under FIFRA guidelines for subchronic studies as it was less than 90 days in duration, histopathological examinations of designated tissues were not performed, lack of clinical chemistry and hematology, and the data were in summary form. Therefore, the study was considered supplemental information.

Sprague-Dawley rats (5/sex/group) were dosed with hydrogen cyanamide (50% w/w) at 5, 10, 20 or 40 mg cyanamide/kg-day by gavage for 28 days (Osheroff, 1988). Food consumption was significantly (P<0.05) decreased in male rats dosed with 20 mg/kg-day (87% of control) and 40 mg/kg-day (75% of control). Significant (P<0.05) decrements in body weight gain were observed in both male and female rats dosed with 20 mg/kg-day (70% and 64% of control, respectively) and 40 mg/kg-day (47% and 43% of control, respectively). Examination of the hematology revealed significant (P<0.05) declines in red blood cell count (91% of control), hemoglobin (84% of control), hematocrit (86% of control), and mean cell hemoglobin (92% of control) at 40 mg/kg-day in males, and in hemoglobin (92% of control), and hematocrit (93% of control) in 40 mg/kg-day females. Blood levels of thyroxine were significantly (P<0.05) depressed in males (74% of control) at 40 mg/kg-day, and thyroid-stimulating hormone levels were significantly (P<0.05) elevated (150% of control). Blood urea nitrogen was significantly (P<0.05) elevated (173% of control) in males at 40 mg/kg-day. Histopathological examination of the thyroid in male rats revealed follicular cell hyperplasia, decreased colloid, and small, closely-packed follicles at 10, 20, and 40 mg/kg-day in all animals. Small and closely-packed follicles (2/5 rats), and decreased colloid (1/5) were observed at 5 mg/kg-day. The LOEL for thyroid toxicity was 5 mg/kg-day. Bile duct hyperplasia in the liver was observed in males at 10, 20, and 40 mg/kg-day. The NOEL for bile duct hyperplasia was 5 mg/kg-day. The study was unacceptable under FIFRA guidelines for subchronic studies as it was less than 90 days in duration, and the number of animals utilized was inadequate. Therefore, the study was considered supplemental information.

Intraperitoneal - rat

Male Wistar rats were given hydrogen cyanamide (prepared from Colme; 6 g cyanamide/100 ml) at 8 and 16 mg/kg-day intraperitoneally with or without ethanol for up to 27 weeks (Guillen and Vazquez, 1984). After 7 weeks of treatment, hepatocytes in rats receiving hydrogen cyanamide exhibited morphological changes consisting of cytoplasmic homogeneous areas, made up of glycogen disposed in *a*-granules and smooth endoplasmic reticulum tubules. In the 13th week of cyanamide treatment, hepatocyte inclusion bodies similar to those described in alcoholics treated with cyanamide were reproduced in the rat at both doses whether or not the rat received alcohol. The inclusion bodies consisted of round, well-demarcated cytoplasmic areas containing a large amount of glycogen dispersed in *B*-granules, lipid droplets and secondary lysosomes. Initially, hepatocytes bearing inclusion bodies were located principally in the periportal areas. In histological sections taken from rats treated for longer periods of time, the lesion appeared to progress toward the center of the lobules. "Inclusion bodies may eventuate in hepatitis, portal fibrosis, and occasionally cirrhosis" (Guillen and Vasquez, 1985). The study was unacceptable under FIFRA guidelines for subchronic studies due to choice of route of exposure, duration of study, too few animals, inadequate dosing regime, limited pathology, lack of clinical chemistry and hematology, and the study was in summary form. Therefore, the study was considered supplemental information.

Diet - Mouse

Crl:CD-1(ICR)BR mice were given hydrogen cyanamide (49% w/v) in drinking water for 6 weeks (Goodyer, 1986). The dosage, based on water consumption, was 0, 26, 72.3, or 171.1 mg cyanamide/kg-day for males, and 0, 37.4, 88.9 or 204.4 mg cyanamide/kg-day for females. No treatment related clinical signs were observed. Water consumption by high dose males and females was significantly ($P<0.05$) reduced (29 - 51%) compared to controls. Body weight was slightly depressed (90% of control) in the high dose males. No gross pathological changes were observed. The study was unacceptable under FIFRA guidelines for subchronic studies due to the inadequate duration, lack of histopathology, clinical chemistry and hematology, and the data were presented in summary form. Therefore, the study was considered supplemental information.

Gavage - Dog

Beagle dogs (4/sex/dose) were given aqueous cyanamide (50% w/v) at 0, 0.6, 2 or 6 mg cyanamide/kg-day seven days a week by gavage for 90 days (Til *et al.*, 1982). Both the absolute and relative testicular weights (61% and 65% of control, respectively) were significantly ($P<0.05$) less in dogs receiving 6 mg/kg-day compared to controls. Histopathological examination of the testes revealed reduced spermatogenesis or atrophy in all four of the high-dose males. Three males in each of the other dose groups had signs of testicular atrophy and/or reduced spermatogenesis. There was no NOEL for testicular toxicity. The LOEL for testicular effects was 0.6 mg/kg-day. The thyroids also seemed to be affected as thyroxine levels were significantly ($P<0.05$) decreased (62% of control) at the end of the study in males dosed with 6 mg/kg-day. No histopathological changes were reported in the thyroid or liver. However, plasma albumin and SGOT (chemical indicators of liver toxicity) were significantly ($P<0.05$) decreased (93% and 87% of control, respectively) at the end of the study in females dosed with 6 mg/kg-day. The NOEL for thyroid toxicity was 2 mg/kg-day. The study was acceptable under FIFRA guidelines for subchronic studies.

D. CHRONIC TOXICITY/ONCOGENICITY

Summary. Hydrogen cyanamide was not oncogenic in the rat, but chronic exposure caused thyroid toxicity (reduced colloid and the formation of micro-follicles, reduced T_3 and thyroxine levels in the plasma). The NOEL for thyroid toxicity in the rat was 1 mg/kg-day. Hydrogen cyanamide was oncogenic in the mouse, causing a significant, dose-related increase in benign granulosa-theca tumors in the ovary. In addition, hydrogen cyanamide caused nephrotoxicity (fibrosis and scarring, atrophic/basophilic tubules, and vacuolar degeneration and necrosis), chronic cystitis of the urinary bladder, hepatotoxicity (biliary proliferation and centrilobular hypertrophy) in the mouse. The NOEL for mouse hepatotoxicity was 29.5 mg/kg-day. The NOEL for mouse nephrotoxicity and chronic cystitis was 13.7 mg/kg-day. In the dog, chronic oral exposure to hydrogen cyanamide caused thyroid toxicity (lower thyroxine levels), changed clinical chemistry indicating reduced over-all metabolism, testicular effects (neutrophil infiltration of testes, oligospermia), and clinical signs (tremors and excessive salivation). The NOEL for these effects in dogs was 0.2 mg/kg-day.

Gavage - Rat

Aqueous hydrogen cyanamide (50% w/w of active ingredient) at 0, 2.5, 7.5 or 30 mg/kg-day was administered by gavage to Sprague-Dawley rats (20/sex/dose) for 16 weeks (Osheroff, 1991). In the 17th week the dosages were reduced to 0, 1, 2.5 and 7.5 mg/kg-day due to deterioration in the general health (hunched posture and tremors during weeks 9-17) of the highest dosage animals. The study lasted a total of 104 weeks. Decrement in body weight was significant ($P<0.05$) at the high dosage in both males (68% of control) and females (84% of control) at the end of the study. Examination of the blood chemistry revealed reduced plasma levels of triiodothyronine and thyroxine in males and females at the end of the study (Table 2). However, levels of thyroid-stimulating hormone (TSH) were not affected in either males or females.

Thyroid toxicity was also indicated histologically by reduced colloid and the formation of micro-follicles in the gland itself. The NOEL for thyroid toxicity (reduced triiodothyronine levels and histopathological changes) was 1 mg/kg-day. No changes in clinical chemistry indicative of liver damage or histopathological changes in the liver were reported. This study was acceptable under FIFRA guidelines.

Table 2 - Incidence of thyroid histopathological changes and alterations in related blood chemistry parameters in rats due to exposure to hydrogen cyanamide via gavage for 2 years (Osheroff, 1991).

	Male Dosage (mg/kg-day)				Female Dosage (mg/kg-day)			
Parameter	0	1	2.5	7.5	0	1	2.5	7.5
Blood Chemistry[a]								
N =	9	12	14	13	14	13	8	13
Triiodothyronine (ng/dl)	89.8	66.9	60.2	44.9	81.0	71.8	67.8	61.7
(% of control)	(100)	(74)	(67)*	(50)*	(100)	(89)	(84)	(76)*
Thyroxine (*u*g/dl)	3.3	3.4	3.3	2.3	2.4	2.3	2.5	2.1
(% of control)	(100)	(103)	(100)	(70)*	(100)	(96)	(104)	(88)
TSH (ng/ml)	3.4	2.4	2.2	3.0	3.1	2.7	2.2	3.4
(% of control)	(100)	(71)	(65)	(88)	(100)	(87)	(71)	(110)
Thyroid Histology								
Reduced colloid	0/20	2/20	7/20	17/20	3/20	1/20	5/20	16/20
	(0)	(10%)	(35%)	(85%)*	(15%)	(5%)	(25%)	(80%)*

a/ Mean blood chemistry values.
* Significantly (P<0.05) different from controls by Dunnett's test

Diet - rat

F344 rats (20/sex in controls; 50/sex in test groups) were fed a diet containing calcium cyanamide (63% commercial formulation with 22% calcium oxide and 12% free carbon) at 100 or 200 ppm for males and 100 or 400 ppm for females for 107 weeks (Ulland, 1979). No toxicity or carcinogenicity were observed. The study was considered unacceptable under FIFRA guidelines because of the lack of dietary analyses, no dose justification, no individual data supplied, only two doses used, and another study was conducted in the same room.

Gavage - dog

Aqueous hydrogen cyanamide (50% w/w) at 0, 0.2, 1.0 or 5.0 mg cyanamide/kg-day was administered in water by gavage to beagle dogs (4 dogs/sex/group) for 52 weeks (Osheroff, 1989). Animals in the high dose group exhibited clinical signs such as tremors and excessive salivation beginning in the fifth week of the study. One female at 1 mg/kg-day exhibited excessive salivation.

Hematological parameters revealed a mild, non-regenerative anemia in the high dose dogs. The anemia was slightly microcytic as characterized by the significantly (P<0.05) decreased mean red blood cell

volume (males 88% and females 90% of controls), mean red blood cell hemoglobin (85% and 87% of controls), and mean red blood cell hemoglobin concentration (97% and 97% of controls). Thyroxine levels were significantly ($P<0.05$) lower in males (56% of control) and females (59% of control). Total cholesterol was elevated (males 139% and females 141% of control). The basal creatinine level was significantly ($P<0.05$) reduced (66% of control) in females. Albumin and glucose levels were depressed in females (78% and 82% of control, respectively). The blood urea nitrogen (BUN) level was significantly ($P<0.05$) reduced in females (53% of control). SGOT (77% of control) and SGPT (56% of control) were decreased in females. At the mid-dose (1 mg/kg-day), the mean red blood cell volume was significantly ($P<0.05$) reduced (95% of controls in males, and 93% of controls in females); mean cellular hemoglobin was significantly ($P<0.05$) diminished (94% of controls in males, and 93% of controls in females), and total cholesterol levels were elevated in females (150%).

At the high dose (5 mg/kg-day), histopathological changes were reported in the liver, spleen, thymus, and testes. There was an increased incidence and/or severity of brown pigment in the Kupffer cells, and very small choleliths were present in the gall bladder of three high dose dogs. Increased extramedullary hematopoiesis was present in splenic sections from two high dose males. All of the males and one of the females at the high dose exhibited atrophy of the thymus. Three of the high dose males exhibited inflammation of the testes and/or decreased spermatogenesis. The presence of immature sperm forms and neutrophil infiltration was observed in one dog at the mid-dose. The NOEL for thyroid toxicity, testicular effects, clinical signs, and hematological changes was 0.2 mg/kg-day. The study was acceptable under FIFRA guideline requirements.

Dietary - Mouse

B6C3F1 mice were fed on a diet containing calcium cyanamide (63% commercial formulation with 22% calcium oxide and 12% free carbon) at 0, 500 or 1000 ppm for 100 weeks (Ulland, 1979). A significant ($P<0.05$), dose-related increase in hemangiosarcomas was observed in males (controls, 1/20; 500 ppm, 5/50; 1000 ppm, 10/50). The study was unacceptable under FIFRA guidelines due to lack of dietary analyses, no individual data, no body weights, and seven other studies [(2-chloroethyl)trimethylammonium chloride; 2,4-diaminotoluene; lead dimethyldithiocarbamate; N-nitrosodiphenylamine; phthalmide; piperonyl sulfoxide; 2,4,5-trimethylaniline] were conducted in the same room.

CD-1 (ICR) BR mice (60/sex/group) received hydrogen cyanamide (49.9% w/w) in the drinking water at 0, 70, 200 or 600 ppm (approximately 10.7, 29.5 or 75.6 mg/kg-day for males; 13.7, 35.2, 101 mg/kg-day for females from water consumption data) for up to 104 weeks (Goodyer, 1990). During the first several months of the study, water intake by both sexes in the 600 and 200 ppm groups were reduced by 30% and 20%, respectively. Decrements in body weights were noted in males (9%) and females (7%) at 600 ppm. Survival to the end of the study was significantly ($P<0.05$) less in 600 ppm females (24) compared to controls (37), and 600 ppm males (14) compared to controls (24). However, the lack of survivability could not be attributed to any single cause. Nephrotoxicity (fibrosis and scarring, atrophic/basophilic tubules, and vacuolar degeneration and necrosis), and chronic cystitis of the urinary bladder were observed in both sexes (Table 3). The NOEL for nephrotoxicity and chronic cystitis was 70 ppm (approximately 13.7 mg/kg-day). Male mice exhibited significant ($P<0.05$) biliary proliferation and a trend toward centrilobular hypertrophy at 600 ppm.

Examination of the data indicated that granulosa-theca tumors in six of the animals (1 at the mid-dose, and 5 at the high dose) were classified as borderline (Greear and Copley, 1993). These tumors have been included in Table 3, and are part of the analysis. A significant ($P<0.01$) increased incidence in granulosa-theca cell tumors were noted in the ovaries of females at 600 ppm (Table 3). However, the incidence of tumors in mid-dose mice was not significantly different from the concurrent controls if a "questionable" (due to tissue necrosis) tumor was included. The incidence of tumors at the high dose was significantly ($P<0.01$) different from the concurrent control group. If the questionable animal were excluded from consideration, the incidence of tumors in the mid-dose group becomes significant. The time to tumor was not different across the dose groups, with the average appearance at 94 weeks. If the incidence of tumors found in historical controls from the same laboratory (Table 4) were used, then the incidence of tumors at both the high and mid-dose were significantly ($P<0.01$ and $P<0.05$, respectively) different from that of

historical controls. Stromal/luteal hyperplasia also exhibited a dose-related trend. The study was acceptable under FIFRA guideline requirements.

Table 3 - Incidence of histopathological changes in mice due to exposure to hydrogen cyanamide in drinking water for at least one year (Goodyer, 1990).

	Male Dosage (mg/kg-day)				Female Dosage (mg/kg-day)			
Tissue	0	10.7	29.5	75.6	0	13.7	35.2	101
Kidney								
Atrophic/basophilic Tubules	28/59 (47%)	28/59 (47%)	29/59 (49%)	36/58 (62%)	20/60 (33%)	19/60 (32%)	15/59 (25%)	25/58 (43%)
Fibrosis/scarring	1/59++ (2%)	1/59 (2%)	1/59 (2%)	7/58* (12%)	0/60++ (0)	1/60 (2%)	1/59 (2%)	6/58* (10%)
Vacuolar Degeneration/Necrosis	0/59++ (0)	1/59 (2%)	5/59* (8%)	12/58** (21%)	3/60++ (5%)	1/60 (2%)	5/59 (8%)	9/58 (16%)
Urinary Bladder								
Chronic cystitis	5/59++ (8%)	7/57 (12%)	17/57** (30%)	39/58** (67%)	6/60++ (10%)	8/59 (14%)	23/55** (42%)	36/57** (63%)
Liver								
Biliary proliferation	1/59+ (2%)	5/57 (9%)	5/57 (9%)	8/58* (14%)	0/60 (0)	2/59 (3%)	2/55 (4%)	2/57 (3%)
Centrilobular hypertrophy	0/59++ (0)	0/57 (0)	1/57 (2%)	3/58 (5%)	3/60 (5%)	1/59 (2%)	1/55 (2%)	5/57 (9%)
Adenoma/carcinoma	18/59 (31%)	11/57 (19%)	7/57 (12%)	8/58 (14%)	0/60 (0)	0/59 (0)	1/55 (2%)	1/57 (2%)
Ovary								
Stromal/Luteal hyperplasia	-	-	-	-	16/60+ (27%)	12/59 (12%)	13/60 (22%)	22/58 (38%)
Granulosa-theca tumor	-	-	-	-	3/60++ (5%)	1/59 (2%)	7/60 (12%)	13/58** (22%)

* Significantly different (P<0.05) from concurrent control by Fishers Exact test.
** Significantly different (P<0.01) from concurrent control by Fishers Exact test.
\+ Significant (P<0.05) for trend (Peto's trend analysis).
++ Significant (P<0.01) for trend (Peto's trend analysis).

Table 4 - Hazleton UK historical control granulosa-theca tumor data from Crl: CD-1(ICR)BR mice (Goodyer, 1991).

Study Year	Duration (weeks)	No. Animals	Incidence (%)
1986	80	51	0
1986	104	62	2
1987	104	51	6
1987	104	51	0
1987	104	51	0
1987	104	51	2
1987	104	51	0
1988	78	102	1
1988	83	102	1
1988	80	51	0
1989	104	51	0
1989	104	50	2
1989	94	51	0
1990	80	51	2
1990	80	51	0

E. GENOTOXICITY

Summary. Hydrogen cyanamide was not mutagenic in the Ames test, and did not stimulate unscheduled DNA synthesis *in vitro*. It did induce chromosomal aberrations in Chinese hamster cells *in vitro*. However, it did not produce micronucleus formation *in vivo*. Thus, the genotoxic potential of hydrogen cyanamide is considered equivocal.

Gene Mutation

Hydrogen cyanamide (53% w/v) at 0.02, 0.04, 0.08, 0.17, 0.42, 0.84, 1.69 and 2.54 mg cyanamide/plate was tested with *Salmonella* strains TA1535, TA1537, TA1538, TA98 and TA100, two trials in triplicate, with and without S9 (rat liver) activation (Jagannath, 1987). There was no reported increase in the reversion rate in either trial, with or without activation. The reversion rate decreased at the highest concentration. The study was considered acceptable to DPR. The acceptability of the genotoxicity studies is based on the Toxic Substances Control Act guidelines (Federal Register, 1985).

Microsome activated *Salmonella* mutagenicity for calcium cyanamide and cyanamide were tested at 0 to 1000 *u*g/plate in triplicate, with and without S9 (rat liver) activation (Willems, 1978). No increase in the reversion rate was reported. The study was considered unacceptable to DPR because the high concentration was not justified, there was no repeat trial to confirm negative results, and no individual plate counts.

Calcium cyanamide (>96% purity) at 0, 33.3, 100, 333.3, 1000 and 3333.3 *u*g/ml, with and without rat liver and hamster liver S9 activation was tested in *Salmonella* strains TA98, TA100, TA1535 and TA1537 (Haworth *et al.*, 1983). Although reversion rates in TA1535 seemingly doubled with hamster liver activation, there was no dose response and precipitates were reported in the plates associated with increased reversion rates. The study was not acceptable to DPR as it was in summary form.

Structural Chromosomal Aberrations

Chinese hamster ovarian cells were exposed *in vitro* to hydrogen cyanamide (53% w/v in aqueous solution; specific gravity 1.06) at 0, 42.4, 56.5, 141 or 283 *u*g/ml for 17.5 hours without activation (Ivett, 1987a). With S9 (rat liver) activation, CHO cells were exposed to 0, 438, 875 or 1310 *u*g/ml for 2 hours treatment (harvested 20 hours later) or 0, 321, or 438 *u*g/ml for 10 hours. There was an increase in aberrations with and without activation. The study was acceptable to DPR.

Hydrogen cyanamide (53% in aqueous solution) was given by oral gavage to ICR mice (5/sex/group) at 0, 31.4, 157.4 or 330.5 mg/kg (Ivett, 1987b). Two males in the high dose group died within 24 hours. The scheduled terminations were at 24, 48 and 72 hours. No positive or negative controls were used at 48 and 72 hours. There was no observed effect on micronuclei formation. The study was acceptable to DPR.

"Kalkstickstoff" [Calcium cyanamide (40-60%), calcium oxide and free carbon] at 0, 10, 50, 250, or 500 *u*g/ml (due to solubility limitations, equal to 330 *u*g/ml) was incubated with Chinese hamster ovary cells for 1 hour (de Raat, 1979). Twenty metaphases per slide were scored. The study was unacceptable to DPR as the number of metaphases scored was inadequate, there was only a single replicate per concentration, and the duration of exposure (1 hour) was not justified.

Calcium cyanamide (23% based on nitrogen content) at 0, 3.06 and 7% (w/v) was tested in Wistar rats (5/sex/group) (Willems, 1979). Each animal was given two 5 ml doses orally, then terminated 6 hours after the second dose. Four hundred hepatocytes per slide, with 5 slides per animal, were examined. Trenimon was used as the positive control. The study was unacceptable to DPR as no toxicity was reported from the high dose, and there was a single termination time.

Other Genotoxic Effects

Hydrogen cyanamide (53% w/v in aqueous solution) at 0, 6.0, 11.9, 23.8, 47.6, 71.4, 95.2, 143 or 190 *u*g/ml was incubated with rat hepatocytes *in vitro* (Cifone, 1987). The assay was done in triplicate, and a total of 150 nuclei per concentration were scored. There was no indication of unscheduled DNA synthesis. The study was acceptable to DPR.

F. REPRODUCTIVE TOXICITY

Summary. Hydrogen cyanamide by gavage was not reported to cause any significant histomorphic changes in parental rats or offspring associated with the treatments. No reproductive effects of hydrogen cyanamide were reported in a study acceptable under FIFRA. The adult NOEL was 1.25 mg/kg-day for decrement in body weight. There was no NOEL for neonatal pup survival (days 0-4). The LOEL was 1.25 mg/kg-day. In an earlier, unacceptable study, dietary exposure to hydrogen cyanamide was reported to cause atrophic seminiferous tubules and interstitial cell proliferation in rats.

Diet - rat

Wistar rats (24/sex/group) were fed a diet containing cyanamide (49% w/w) at 0, 20, 60, or 180 ppm for two generations with two litters per generation (Koeter *et al.*, 1986). Body weight gains were depressed (8-15%) in both males and females at 180 ppm in both the F_0 and F_1 generations. Relative thyroid weights were significantly ($P<0.05$) increased in F_0 and F_1 males (125% and 131% of controls, respectively), and F_0 and F_1 females (118% and 119% of controls, respectively) at 180 ppm. There were no consistent effects on litter size and number, or on pup growth rate and survival. Although there was no significant effect on relative testicular weights, histopathological examination of the testes of the F_1 generation revealed interstitial cell proliferation at 60 and 180 ppm and atrophic seminiferous tubules at 20, 60, and 180 ppm. No such changes were noted in the F_0 generation males. The LOEL for testicular effects was 20 ppm. Because of a lack of

dietary analyses, the dosage (mg/kg-day) could not be calculated with accuracy. The study was considered unacceptable under FIFRA guidelines because of a lack of individual data, inadequate description of the test material, and a limited number dietary analyses with a problem of stability of the active ingredient.

Gavage - rat

Charles River rats (26/sex/dose in both the F_0 and F_1 generations) were given hydrogen cyanamide (50% w/w) 0, 1.25, 3.75 or 15 mg cyanamide/kg-day by oral gavage for two generations (initial high doses were 7.25 and 30 mg cyanamide/kg-day for 12 weeks; reduced due to significant clinical signs, and decrement in food consumption and weight gain)(Morseth, 1990). Hydrogen cyanamide had no significant effect on fertility or gestation indices in either the F_0 or F_1 generations. A decrement in body weight was observed at the high dosage in males and females in both the F_0 and F_1 generations and the mid-dose males in the F_1 generation (Table 5). The adult NOEL was 1.25 mg/kg-day for statistically significant decrements in body weight in mid- and high dose males in the F_1 generation. Birth weights of pups were not significantly different from one another. However, F_2 pup growth during lactation was significantly ($P<0.05$) less at the high and mid-doses compared to controls. The differences in covariate-adjusted mean pup weights were significant on days 0 (15 mg/kg-day females), 4 pre-cull (3.75 mg/kg-day males and females), 4 post-cull (3.75 mg/kg-day females), 7 (3.75 mg/kg-day males and females), 14 (15 and 3.75 mg/kg-day males; 3.75 mg/kg-day females), and 21 (15 and 3.75 mg/kg-day males and females). Neonatal survival (day 0-4), as indicated by the viability index (# alive at birth/# alive at day 4) was significantly ($P<0.05$) lower in all treatment groups for both the F_1 and F_2 generations, but did not exhibit a dose-response. There was no NOEL for neonatal survival. The LOEL for neonatal survival (day 0-4) was 1.25 mg/kg-day. The treatments did not cause any significant changes in organ weights, organ appearance, or histopathology of the organs in parental stock or offspring. The study was acceptable under FIFRA guideline requirements.

Table 5 - Effects of hydrogen cyanamide by gavage on the body weights of the F_0 and F_1 generations and neonatal survival of rats (Morseth, 1990).

	Dosage (mg/kg-day)			
	0	1.25	3.75	15
Adult Body Weights[a]				
F_0 (males, 24 wks)	613 ± 35	626 ± 31	616 ± 52	491 ± 48**
N =	25	26	26	26
F_0 (females, 18 wks)	432 ± 34	343 ± 46	373 ± 46	299 ± 73*
N =	3	5	4	5
F_1 (males, 24 weeks)	642 ± 69	611 ± 58	595 ± 64*	487 ± 51**
N =	26	26	25	26
F_1 (females, 15 weeks)	313 ± 27	324 ± 29	311 ± 34	272 ± 27*
N =	25	25	21	26
Pup Viability				
F_1 Viability Index (Day 4/ Day 0)	92	83**	88**	84**
F_2 Viability Index (Day 4/ Day 0)	93	87*	82**	81**

a/ Mean ± standard deviation
* Significantly different (P<0.05) from control by Dunnett's test.
** Significantly different (P<0.01) from control by Dunnett's test.

G. DEVELOPMENTAL TOXICITY

Summary. In rabbits, the NOEL for maternal toxicity (significant decrement in weight gain) was 6 mg/kg-day. The NOEL for developmental toxicity (retinal folds) was 2 mg/kg-day. Hydrogen cyanamide by gavage caused an increased incidence of diaphragmatic hernias and depression of fetal body weights in rats. The NOEL for developmental effects in rats was 15 mg/kg-day. The maternal NOEL for clinical signs (hypoactivity, hunched posture, fecal and urine stains, protruding eyes, malocclusion, and chromodacryorrhea) in rats was 5 mg/kg-day.

Gavage - rabbit

Mated New Zealand white rabbits (24-25/group) were dosed by gavage with hydrogen cyanamide (49% w/w) at 0, 2, 5.9 or 17.6 mg cyanamide/kg-day on days 6 through 19 of gestation (Koeter and van Marwijk, 1989). A significant (P<0.05) treatment related decrease in maternal weight gain was observed (Table 6). No significant differences in food consumption were observed. No clinical signs were manifested by the treated animals at any time. Fetal resorption, weights, and survivability from treated animals were not significantly different from controls. A significant (P<0.05) treatment related trend in the incidence of retinal folds was observed. Seven fetuses in 3 litters were affected at the high dose, and seven fetuses in 5 litters were affected at the mid dose. The NOEL for maternal toxicity (significant decrement in weight gain) was 5.9 mg/kg-day. The NOEL for developmental toxicity (retinal folds) was 2 mg/kg-day. The study was acceptable under FIFRA guideline requirements.

Table 6 - Effect of oral doses of hydrogen cyanamide on maternal body weight change and fetal development in the rabbit (Koeter and van Marwijk, 1989).

Parameter	Dosage (mg/kg-day) 0	2	6	18
Mean Maternal BW Change (g) (days 6-19)	109+ (N=23)	120 (N=20)	86 (N=23)	-10* (N=20)
Fetal Retinal Folds				
Bilateral (total fetuses)	1/181+ (1%)	2/144 (1%)	7/149 (5%)*	7/130 (5%)**
Unilateral (total fetuses)	7/181+ (4%)	8/144 (6%)	7/149 (5%)	11/130 (8%)

* Significantly different (P<0.05) from control by Dunnett's test.
** Significantly different (P<0.01) from control by Dunnett's test.
\+ Significant (P<0.05) for trend (Peto's Trend Analysis).

Gavage - rat

Mated, female Sprague-Dawley rats (25/group) were dosed by gavage with hydrogen cyanamide (50% w/w) at 0, 5, 15 or 45 mg cyanamide/kg-day on days 6 through 15 of gestation (Morseth, 1989). A total of 8 dams at 15 and 45 mg/kg-day were hypoactive on the first 2 days of treatment, and other clinical signs (hunched posture, fecal and urine stains, protruding eyes, malocclusion, and chromodacryorrhea) appeared later. The short-term maternal NOEL for clinical signs (hypoactivity) was 5 mg/kg-day. The mean maternal body weight change and food consumption during the dosing period exhibited a significant (P<0.01) dose response (Table 7). At the high dose, the mean fetal body weight was significantly (P<0.05) depressed compared to control. The developmental NOEL was 15 mg/kg-day for reduced fetal weight, and soft tissue malformations. The study was acceptable under FIFRA guideline requirements.

Table 7 - Effect of oral doses of hydrogen cyanamide on maternal body weight change and food consumption, and fetal development in the rat (Morseth, 1989).

Parameter	Dosage (mg/kg-day) 0	5	15	45
Mean Maternal BW Change (g) (days 6-16)	50.2+ (N=25)	40.9* (N=25)	31.9* (N=25)	6.2* (N=25)
Mean Food Consumption (g/kg) (days 6-16)	225.4+	217.7	200.2*	167.6*
Mean Fetal BW (g)				
Males	3.3+ (N=25)	3.2 (N=25)	3.2 (N=24)	2.9* (N=24)
Females	3.2+ (N=25)	3.1 (N=25)	3.0 (N=25)	2.8* (N=24)
Diaphragmatic Hernia				
Fetal incidence	0/174	0/175	0/169	7/163*
Litter incidence	0/25	0/25	0/25	5/24*

* Significantly different (P<0.05) from control by Dunnett's test.
** Significantly different (P<0.01) from control by Dunnett's test.
\+ Significant (P<0.05) for trend (Peto's Trend Analysis).

H. SPECIAL STUDIES

Dermal and Inhalation- Human

Blood and urine samples were collected from 21 men exposed to calcium cyanamide in the workplace and 9 men not exposed to calcium cyanamide (Mertschenk *et al.*, 1993). No significant difference between the two groups was found in plasma levels of TSH, thyroxine, triiodothyronin, thyroxine binding globulin, FSH, LH, or testosterone. The mean concentration of *N*-acetylcyanamide in the urine of exposed men was 0.75 mg/liter before work, and 2.14 mg/liter after an 8 hour work day. The indicated exposure was nearly two orders of magnitude below that which would be expected to affect blood chemistry indicators of thyroid function. None of the laboratory animal data suggests that an effect on reproductive hormone levels would be expected, even at higher doses. The study was considered supplemental information.

III RISK ASSESSMENT

A. HAZARD IDENTIFICATION

Possible adverse effects were indicated in each toxicity category. Selected studies and results are summarized in Table 8. Examination of the application practices for hydrogen cyanamide indicates that it is used only during a four week period in a given year (Appendix B). However, there were indications that at least some of the toxic effects of hydrogen cyanamide are not reversible. Consequently, the occupational exposures to be considered will be acute, seasonal, annual, and lifetime.

Neither acute nor chronic NOELs were established for potential routes of exposure other than oral. Consequently, the MOS calculations presented in this document use the oral NOELs from laboratory animal studies to assess the risk of human exposure to hydrogen cyanamide via all potential routes of exposure. As oral absorption was effectively 100% (Struble, 1992; Gloxhuber *et al.*, 1989), the NOELs for oral administration represent the NOEL for an absorbed dose.

Acute

The toxicological basis for assessing the risks associated with potential short term exposure to hydrogen cyanamide was identified in developmental toxicity studies. In the rat, the maternal LOEL was 15 mg/kg-day with clinical signs (hunched posture, fecal and urine stains, protruding eyes, malocclusion, and chromodacryorrhea) being expressed on the first two days of treatment with a NOEL of 5 mg/kg-day (Morseth, 1989). The lowest acute NOEL was 2 mg/kg-day for retinal folds in rabbit fetuses (Koeter and van Marwijk, 1989). Fetal retinal folds were found in both control and treated rabbits. When the incidence of folds occurring in at least one eye was examined, there was no significant difference between the controls (4%) and any treatment group (8% at the highest dose). Trend analysis indicated a significant ($P<0.05$) trend, but a clear dose response was not evident. When the incidence of retinal folds was reclassified, recording the number of times retinal folds occur in both eyes, then there was a significant ($P<0.05$) difference between the two highest doses (5%) and the controls (1%). Again, there was no clear dose response even though trend analysis indicated a significant ($P<0.05$) trend. At the high dose, the number of litters (3) exhibiting bilateral retinal folds was fewer than at the mid-dose (5), even though the number of affected fetuses was the same. Retinal folds may occur in normal development, or because of fixation artifacts, as well as a result of chemical effects. In this instance the effect was too marginal to be of sufficient toxicological significance to warrant use as a regulatory endpoint. Thus, the NOEL from the developmental study, 5.0 mg/kg-day for maternal clinical signs, was used as the basis for calculation of margins of safety for potential acute exposure to hydrogen cyanamide.

Seasonal (subchronic)

Three aspects were considered in the selection of an appropriate endpoint and NOEL to be used for assessing health risks from seasonal exposure to hydrogen cyanamide. First, the most sensitive toxicological endpoint was sought from subchronic reproductive toxicity studies. Second, the acceptability of the studies according to FIFRA guidelines, or from a scientific viewpoint was considered. Third, the applicability of the duration of the laboratory study to the observed duration of human exposure was examined. The integration of these three factors was necessary to make the risk assessment for seasonal exposure pertinent.

The three toxicity endpoints for hydrogen cyanamide reported in chronic and subchronic studies were liver, thyroid, and testicular toxicity. Hydrogen cyanamide in the diet of rats for 28 days caused hepatotoxicity (hydropic liver cell degeneration, individual liver cell degeneration, enlarged periportal hepatocytes with clumped cytoplasm, and bile duct proliferation), with a LOEL of 4.6 mg/kg-day, the lowest dose tested (Til *et al.*, 1974). Because there was no NOEL, an Estimated-No-Effect-Level (ENEL) was calculated by dividing the LOEL of 4.6 mg/kg-day by an uncertainty factor of 10 (Dourson and Stara, 1985; USEPA, 1987). This default procedure yielded an ENEL (28-day) of 0.46 mg/kg-day. Rats given

Table 8 - Summary of Selected Hydrogen Cyanamide Toxicology Studies

STUDY	SPECIES	ROUTE	EFFECT	LOEL	NOEL	GENOTOXIC	REF[a]
				(mg/kg-day)			
28-day	rat	diet	hepatotoxicity	4.6	-		1
28-day	rat	gavage	hepatotoxicity	10.0	5.0		2
28-day	rat	gavage	thyroid hyperplasia	5.0	-		2
90-day	rat	diet	thyroid toxicity	2.4	0.8		3
90-day	dog	gavage	testicular atrophy	0.6	-		4*
90-day	dog	gavage	thyroid toxicity	6.0	2.0		4*
combined	rat	gavage	thyroid toxicity	2.5	1.0		5*
onco.	mouse	diet	nephrotoxicity	35.2	13.7		6*
onco.	mouse	diet	hepatotoxicity	75.6	29.5		6*
chronic	dog	gavage	thyroid toxicity	1.0	0.2		7*
chronic	dog	gavage	testicular atrophy	1.0	0.2		7*
repro.	rat	gavage	mat. decr. wt. gain	3.8	1.3		8*
repro.	rat	gavage	decr. pup survival	1.3	-		8*
develop.	rat	gavage	mat. clinical signs	15.0	5.0		9*
develop.	rat	gavage	herniated diaphr.	45.0	15.0		9*
develop.	rabbit	gavage	mat. decr. wt. gain	17.6	5.9		10*
develop.	rabbit	gavage	retinal folds	5.9	2.0		10*
mutat.	*Salmonella*	*in vitro*	mutagenicity			-	11**
chrom.	CHO	*in vitro*	aberrations			+	12**
chrom.	mouse	*in vivo*	micronucleus			-	13**
UDS	rat hepat.	*in vitro*	unsched. DNA syn.			-	14**

a/ 1. Til *et al.*, 1974; 2. Osheroff, 1988; 3. Til *et al.*, 1975; 4. Til *et al.*, 1982; 5. Osheroff, 1991; 6. Goodyer, 1990; 7. Osheroff, 1989; 8. Morseth, 1990; 9. Morseth, 1989; 10. Koeter and van Marwijk, 1989; 11. Jagannath, 1987; 12. Ivett, 1987a; 13. Ivett, 1987b; 14. Cifone, 1987.

* Acceptable under FIFRA guidelines.

** Acceptable under TSCA guidelines.

hydrogen cyanamide by gavage for 28 days exhibited bile duct hyperplasia in the liver with a NOEL of 5 mg/kg-day (Osheroff, 1988). Longer term exposures of humans to hydrogen cyanamide have been reported to cause hepatotoxicity (Vazquez and Cervera, 1980; Thomsen and Reinicke, 1981; Vazquez *et al.*, 1983a,b; Villegas, 1984; Bruguera *et al.*, 1986), but these observations were complicated by the fact the human subjects were alcoholics.

Thyroid toxicity, as indicated by decreased plasma thyroxine levels, was observed in a 90-day dog study (Til *et al.*, 1982). The NOEL for thyroid toxicity in the dog was 2 mg/kg-day. Rats exposed to hydrogen cyanamide by gavage for 28 days displayed thyroid follicular cell hyperplasia, decreased colloid, and small, closely-packed follicles, with a LOEL of 5 mg/kg-day (Osheroff, 1988). The 28-day ENEL for thyroid toxicity (using the default procedure described above) was 0.5 mg/kg-day. However, rats exposed to hydrogen cyanamide in the diet for 90 days had a LOEL for thyroid toxicity (small follicular lumens without colloid, separated by proliferating epithelial cells and interfollicular cells) of 2.4 mg/kg-day, with a NOEL of 0.8 mg/kg-day (Til *et al.*, 1975). Accordingly, the actual 28-day no effect level for thyroid toxicity probably lies somewhere between 0.8 mg/kg-day (the 90-day NOEL) and 5 mg/kg-day the 28-day LOEL.

The most sensitive endpoint appeared to be testicular atrophy and oligospermia in the dog, with a LOEL of 0.6 mg/kg-day occurring after a 90-day dose regime (Til *et al.*, 1982). Because a NOEL was not established, the ENEL would be 0.06 mg/kg-day. However, a 90-day ENEL of 0.06 mg/kg-day was less than the observed NOEL of 0.2 mg/kg-day reported in dogs after a 1 year exposure (Osheroff, 1989). Consequently, the actual NOEL at 90 days is likely to be below 0.6 mg/kg-day, but above the 1-year NOEL of 0.2 mg/kg-day. Testicular atrophy was also reported in a rat reproduction study with a LOEL of 20 ppm (Koeter *et al.*, 1986); however, the dosage could not be estimated with accuracy.

Thus far, this discussion has centered on determining the most sensitive toxicological endpoint in subchronic studies, regardless of the quality of the study. Under FIFRA guidelines series 82-1, acceptable subchronic toxicity studies should be 90 days in duration, conducted with good laboratory practices, have a specified number of animals of both sexes in the study, analysis of dosing material, and report on hematology, blood chemistry, dietary consumption, weights, gross and histopathology of various body organs (USEPA, 1984). Only the 90-day subchronic dog study met all of the FIFRA guidelines. This does not mean that the other studies were scientifically invalid, only that some of the FIFRA criteria were not met by those studies. To be applicable for calculating margins of safety for the human exposure situation, the study would be ideally 28 days in duration. Yet, studies of this duration do not meet the FIFRA criteria for subchronic toxicity studies.

The duration of animal exposure in the rat reproduction study was also approximately 90 days (Morseth, 1990). The LOEL for neonatal survival (day 0-4) in both the F_1 and F_2 generations was 1.25 mg/kg-day. Because the magnitude (incidence) of the effect was small, and the slope of the dose response was fairly shallow (-0.29 to -0.6), an uncertainty factor of 3 was used to derive an ENEL of 0.42 mg/kg-day (USEPA, 1987). The ENEL for neonatal survival (0.42 mg/kg-day) falls in the range of the expected 90-day NOEL for testicular atrophy in dogs (between 0.6 and 0.2 mg/kg-day), and is not substantially different from the 28-day ENEL for liver toxicity in rats. Consequently, the ENEL of 0.42 mg/kg-day for neonatal survival in the rat will be used to calculate the margins of safety for seasonal exposure to hydrogen cyanamide.

Chronic

Even though the use-season for hydrogen cyanamide is limited to a 4-week period, it is possible that some of the toxic effects may not be reversible. Testicular atrophy, observed in dogs in a 90-day subchronic study (Til *et al.*, 1982), was also present in dogs in a chronic exposure study (Osheroff, 1989). In the absence of data to the contrary, it was assumed that testicular atrophy induced by short term exposure to hydrogen cyanamide may persist. Consequently, the NOEL (1-year) for testicular atrophy, thyroid toxicity (lower thyroxine levels, clinical chemistry indicating reduced over-all metabolism), and

clinical signs (tremors and excessive salivation) in dogs (0.2 mg/kg-day) was used to establish margins of safety for potential long term effects from occupational exposure to hydrogen cyanamide.

Lifetime

The weight of evidence suggests that hydrogen cyanamide is weakly oncogenic. The oncogenicity study in rats did not reveal any tumors associated with hydrogen cyanamide toxicity (Osheroff, 1991). In a mouse carcinogenicity study, a calcium cyanamide formulation caused an apparent increase in hemangiosarcomas (Ulland, 1979). However, the study was uninterpretable because seven other compounds, three of which were B2 carcinogens (2,4-diaminotoluene; lead dimethyldithiocarbamate; *N*-nitrosodiphenylamine- NTP, 1991), were tested in the same room at the same time. In a more recent study, hydrogen cyanamide caused a dose-related increase in ovarian granulosa-theca tumors in mice from week 79 onward (Goodyer, 1990). At the high dose, the tumor incidence was significantly greater ($P<0.01$) than both concurrent and historical controls. Such tumors in humans also appear late in life, but human granulosa-theca tumors slowly progress toward malignancy (Kurman, 1987). In humans the tumors generally remain focal in the ovary.

The genotoxicity data for hydrogen cyanamide were equivocal. Chromosomal aberrations were induced *in vitro* (Ivett, 1987a). However, all other genotoxicity data were negative. Thus, the weight of evidence for developing a potency factor for the occurrence of granulosa-theca tumors was weak. Nonetheless, the fact that there was unmistakeable, significant, oncogenic dose response in the mouse cannot be ignored. Consequently, a linear multistage model (Global 86) was used to estimate the carcinogenic potency of hydrogen cyanamide in mice. An interspecies scaling factor, (human body weight/mouse body weight) $^{1/4}$, was used to adjust the potency factor for the difference between humans and mice. The maximum likelihood estimate (MLE) for human cancer potency was 0.007 $(mg/kg\text{-}day)^{-1}$, with an upper bound (95% confidence level) of 0.02 $(mg/kg\text{-}day)^{-1}$ (Appendix B).

B. EXPOSURE ASSESSMENT

Hydrogen cyanamide is applied to dormant, trimmed grapevines, but not directly to grapes. Based on the physical/chemical properties of hydrogen cyanamide, environmental fate studies, and grape residue studies, residues of hydrogen cyanamide are not expected to be found in grapes. Consequently, exposure through the dietary route is not expected, and only occupational exposures were considered.

The label on technical grade hydrogen cyanamide requires workers handling the material to wear waterproof protective clothing, rubber gloves and rubber boots. Loaders must wear waterproof aprons, and workers in areas of spray drift must wear a National Institutes of Safety and Health (NIOSH) approved organic vapor respirator. The label prohibits the consumption of alcoholic beverages prior to, during, and 24 hours following the application. This precaution is to prevent the adverse reaction to alcohol which hydrogen cyanamide can provoke (Armstrong and Kerr, 1956; Smith *et al.*, 1957; Armstrong, 1957; Smith *et al.*, 1959; Marconi *et al.*, 1960; Mukasa *et al.*, 1964).

The studies and data which form the basis for estimating worker exposure are described by Formoli *et al.*, 1993. These estimates were based on worker monitoring data. Patch monitoring was conducted during the 1987-1988 application season. Calculated exposures from the 1987-1988 application season were adjusted to reflect current protective equipment required by the label. Urine samples were collected from mixer/loaders, applicators, and supervisors during the 1988-1989 and 1992-1993 application seasons. The absorbed dosage of hydrogen cyanamide was estimated from urinary concentrations of *N*-acetylcyanamide, a metabolite. The calculated absorbed daily dosages (ADDs) for each year, plus the weighted average ADDs are presented in Table 9.

Table 9 - Mean Absorbed Daily Dosages for Agricultural Workers from Potential Exposure to Hydrogen Cyanamide[a]

	ADD (*u*g/kg-day)			
	1987-88[a]	1988-89[b]	1992-93[b]	Average[c]
Mixer/Loader (N=)	15 (7)	6 ± 5 (8)	16 ± 25 (4)	11.6 ± 4.7 (19)
Applicator (N=)	3 (39)	5 ± 5 (22)	14 ± 4 (11)	5.3 ± 3.8 (72)
Supervisor (N=)	-	3 ± 9 (5)	2 ± 38 (4)	2.6 ± 0.5 (9)

a/ Mean absorbed daily dosage estimated from patch monitoring data, adjusted for wearing appropriate protective clothing (from Table 4, Formoli *et al.*, 1993).
b/ Mean, ± standard deviation, absorbed daily dosage of hydrogen cyanamid estimated from urinary monitoring data (from Table 4, Formoli *et al.*, 1993).
c/ Weighted average, ± standard deviation, of the studies for all three years.

The actual number of days when hydrogen cyanamide was applied during the 60-day season ranged from 3 to 30 days, with an estimated 17 days to spray the average size vineyard. The allowed workdays each season (limits mandated on the label) for mixer/loaders is 12 days, for applicators, 26 days, and for mixer/loader/applicators, 30 days (no more than 12 days as a mixer/loader). The average absorbed daily dosage for mixer/loaders was 11.6 ± 4.7 *u*g/kg-day, for applicators 5.3 ± 3.8 *u*g/kg-day, and for supervisors 2.6 ± 0.5 *u*g/kg-day (Table 10). The 60-day mean seasonal absorbed daily dosage ranged from to 1.3 *u*g/kg-day for supervisors to 3.9 *u*g/kg-day for mixer/loader/applicators. The mean annual absorbed daily dosage ranged from 0.2 *u*g/kg-day for supervisors to 0.7 *u*g/kg-day for mixer/loader/applicators. The estimates for mean lifetime absorbed daily dosage ranged from 0.1 *u*g/kg-day for supervisors to 0.4 *u*g/kg-day for mixer/loader/applicators.

Under previously granted Section 18 Emergency Exemptions for the use of hydrogen cyanamide, a buffer zone of 300 feet had been required by DPR to protect for potential non-occupational exposure to drifting pesticide. The estimated absorbed daily dosage of a person working in a nearby field, exposed for 1 hour a day to drift, was 7.5 *u*g/kg-day at 100 feet, 4.1 *u*g/kg-day at 200 feet, and 2.9 *u*g/kg-day at 300 feet (Table 5; Formoli *et al.*, 1993). No data are available which would suggest that a bystander would be incidentally exposed to drift on a lifetime, annual, or seasonal basis.

Table 10 - Mean Absorbed Dosages for Agricultural Workers from Potential Daily, Seasonal, Annual and Lifetime Exposure to Hydrogen Cyanamide[a]

Work Task	Sample #	ADD[b] (ug/kg-day)	SADD[c] (ug/kg-day)	AADD[d] (ug/kg-day)	LADD[e] (ug/kg-day)
Mixer/Loader[f]	19	11.6 ± 4.7	2.3	0.4	0.2
Applicator[g]	72	5.3 ± 3.8	2.3	0.4	0.2
Mixer/Loader/Applicator		-	3.9	0.7	0.4
Supervisor[h]	9	2.6 ± 0.5	1.3	0.2	0.1

a/ Data derived from Table 9.
b/ Weighted average Absorbed Daily Dosage of hydrogen cyanamide.
c/ Seasonal Absorbed Daily Dosage, assumes only 12 days of use for mixer/loaders, 26 days of use for applicators, and 30 days of use for mixer/loader/applicators and supervisors during a 60 day period.
d/ Annual Average Daily Dosage, assumes 12 days of use for mixer/loaders, 26 days of use for applicators, and 30 days of use for mixer/loader/applicators and supervisors during the 365-day year.
e/ Lifetime Average Daily Dosage, assumes 40 years of working during a 70 year lifetime.
f/ A closed system is used for mixing/loading and mixer/loaders wore rubber suits, rubber gloves, respirator, rubber boots, goggles, and rubber aprons on top of normal work clothing.
g/ Applicators wore rubber gloves, respirator, goggles, rubber boots, and rubber suits on top of normal work clothing.
h/ Supervisors wore the same protective clothing when involved in handling.

C. RISK CHARACTERIZATION

The margins of safety (MOS) corresponding to various potential occupational exposures to hydrogen cyanamide are presented in Table 11. A margin of safety is defined as the ratio of the absorbed dosage of hydrogen cyanamide which produced no effect (NOEL) in a human or laboratory animal study to the absorbed dosage of hydrogen cyanamide to which a specific population subgroup is potentially exposed. MOSs for the mean potential acute occupational exposures, based on the NOEL of 5 mg/kg-day for clinical signs (Morseth, 1989), ranged from 431 (mixer/loaders) to 1,923 (supervisors). If the 95 percent confidence limit of short-term exposure (mean plus two standard deviations) were considered for each of the job categories, the MOSs would range from 238 (mixer/loaders) to 1,389 (supervisors). MOSs for potential drift exposure of laborers working nearby ranged from 667 at 100 feet to 1,724 at 300 feet. The MOSs for mean potential seasonal exposure, based on an ENEL of 0.42 mg/kg-day for neonatal survival in rats (Morseth, 1990), ranged from 108 (mixer/loader/applicators) to 323 (supervisors). MOSs for mean potential annual occupational exposures, based on a NOEL of 0.2 mg/kg-day for testicular atrophy, thyroid toxicity and clinical signs in dogs (Osheroff, 1989), ranged from 286 (mixer/loader/applicators) to 1,000 (supervisors). The maximum likelihood estimates for additional lifetime risks of cancer ranged from 1×10^{-6} (supervisors, mixer/loaders, applicators) to 3×10^{-6} (mixer/loader/applicators), based on ovarian granulosa-theca tumors observed in the mouse (Goodyer, 1990). The 95% upper confidence limit on the maximum likelihood estimates for additional lifetime risks of cancer ranged from 2×10^{-6} (supervisors) to 8×10^{-6} (mixer/loader/applicators).

Table 11 - Margins of Safety for Mean Potential Daily, Seasonal, and Annual Absorbed Dosages of Hydrogen Cyanamide, and the Maximum Likelihood Estimate for Additional Lifetime Risk of Cancer for Agricultural Workers[a]

Work Task	ADD MOS[b]	SADD MOS[c]	AADD MOS[d]	Lifetime Risk MLE[e]	Lifetime Risk UB[f]
Mixer/Loader	431	183	500	1×10^{-6}	4×10^{-6}
Applicator	943	183	500	1×10^{-6}	4×10^{-6}
Mixer/Load/Applicator	-	108	286	3×10^{-6}	8×10^{-6}
Supervisor	1,923	323	1,000	1×10^{-6}	2×10^{-6}
Bystander					
100 feet	667				
200 feet	1,220				
300 feet	1,724				

a/ Dosages from Table 10.
b/ Based on a NOEL of 5 mg/kg-day for clinical signs in rats (Morseth, 1989).
c/ Based on an ENEL of 0.42 mg/kg-day for pup survival in the rat (Morseth, 1990).
d/ Based on a 1-yr NOEL of 0.2 mg/kg-day for testicular atrophy, thyroid toxicity (lower thyroxine levels, clinical chemistry indicating reduced over-all metabolism), and clinical signs (tremors and excessive salivation) in the dog (Osheroff, 1989).
e/ Based on the maximum likelihood estimate of human carcinogenic potency- 7×10^{-3}, for ovarian granulosa-theca tumors observed in the mouse (Goodyer, 1990).
f/ Based on the 95th upper bound estimate of human carcinogenic potency- 2×10^{-2}, for ovarian granulosa-theca tumors observed in the mouse (Goodyer, 1990).

V. RISK APPRAISAL

Risk assessment is a process used to evaluate the potential for exposure and the likelihood that the toxic effects of a substance, often characterized only in laboratory animals, may occur in humans under the specific exposure conditions. Every risk assessment has inherent limitations on the application of existing data to estimate the potential risk to human health. Therefore, certain assumptions and extrapolations are incorporated into the hazard identification, dose-response assessment, and exposure assessment processes. This, in turn, results in uncertainty in the risk characterization, which integrates all the information from the previous three processes. Qualitatively, risk assessment for all chemicals has similar types of uncertainty. However, the degree or magnitude of the uncertainty varies depending on the quality and availability of the toxicological data, and the data for the exposure scenarios being assessed.

A margin of safety of at least 100, when based on laboratory animal studies, is generally considered adequate for protection against the potential toxicity of hydrogen cyanamide. However, the number 100 is only a benchmark. This benchmark of 100 assumes that the average human is 10 times more sensitive to hydrogen cyanamide than are laboratory animals, and includes an additional uncertainty factor of 10 for potential variability in individual human response (Davidson *et al.*, 1986; Dourson and Stara, 1983, 1985; USEPA, 1986). In the absence of scientific evidence to the contrary, the effects of hydrogen cyanamide observed in laboratory animals are expected to occur in humans at similar doses. Specific areas of uncertainty associated with this risk assessment for hydrogen cyanamide are delineated in the following discussion.

Toxicological Data

The selection of an appropriate NOEL to gauge the risks of seasonal (30 days) exposure to hydrogen cyanamide was difficult. An ENEL of 0.42 mg/kg-day for neonatal survival in the rat (Morseth, 1990) was calculated by assuming the NOEL was a factor of 3 less than the LOEL. The actual NOEL may be somewhat higher or lower than this ENEL. In addition, the duration of laboratory animal exposure (98 days) was three times longer than the maximum duration of expected human exposure (30 days). Most laboratory data indicate that the dose of a toxin required to elict a specific effect declines with the duration of continuous administration that toxin (USEPA, 1986). Consequently, a 30-day NOEL for neonatal rat survival is likely to be greater than the 98-day ENEL (0.42 mg/kg-day) selected for calculating the MOS for potential seasonal exposure.

It was assumed that some of the toxic effects, such as thyroid toxicity, and testicular atrophy may not be reversible. If these toxic effects are reversible, then the MOSs for chronic exposures, based on amortized seasonal exposures, are probably much greater. Likewise, the application of a potency factor to estimates of lifetime exposure depends upon the accrual of the oncogenic effects of hydrogen cyanamide. If those effects are repairable, then the additional lifetime risk of cancer will likely be less than presented in this document.

Exposure Data

The estimate of absorbed dosage was based on the assumption that the *N*-acetylcyanamide in the collected urine represented 40% of the absorbed dosage (Formoli *et al.*, 1993). The metabolic study on which this assumption was based indicated there was a degree of variability ($\pm$ 11%) in the amount of absorbed material converted to the metabolite in humans (Gloxhuber *et al.*, 1989). Consequently, the actual exposure may be somewhat higher or lower than the estimated exposure. The small number of workers included in the study also contributes to the uncertainty of the short-term exposure estimates. To protect for possible underestimation of short-term exposure, the MOSs for the 95% upper confidence limit on potential short-term exposures were calculated. These MOSs were also adequate.

The variability in the duration of the use season also contributes to the uncertainty of the exposure estimate. As indicated, the use period can vary from 3 to 30 days, with an average of 17 days. The current label requires mixer/loaders to work no more than a maximum of 12 days, and applicators may not work more than 26 days. The MOSs for the SADDs and AADDs are likely to be greater for most workers. Likewise, the LADD is probably a gross overestimate, as it assumes that the same people will work in similar vineyards for 40 years receiving the same seasonal exposure each of those years.

VI. CONCLUSIONS

Using current toxicity data and estimates from worker monitoring information on hydrogen cyanamide, the calculated margins of safety (MOSs) for potential acute, seasonal, and annual exposures were considered adequate. The maximum likelihood estimate for additional lifetime risk of cancer ranged from 1×10^{-6} to 4×10^{-6} and were considered acceptable because of the overestimation of lifetime occupational exposure, and the weak weight of evidence supporting the possible occurrence of carcinogenicity. The 95% upper confidence limit on the maximum likelihood estimates for additional lifetime risk of cancer ranged from 2×10^{-6} to 8×10^{-6} and were also considered acceptable for the same reasons.

Likewise, MOSs for potential acute exposure of bystanders to drift, at distances from 100 to 300 feet from the site of operations, were adequate.

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IX. APPENDICES

APPENDIX A

Summary of Toxicology Information

TO: James Herota, Registration Specialist
Pesticide Registration Branch

FROM: Medical Toxicology Branch **Date:** 9/8/93 (revised)
12/28/87 (original)

PRODUCT REGISTRATION RECOMMENDATION SHEET

Formulated Product Name: Dormex
Chemical Code #: 2238 **ID #:** 135613N
EPA Reg. #: **SB 950 #:** New A.I.
Document #'s: 50660-003, 004, 012, 020, 035-039, 040, 043-044, 048, 071-072, 075-077, 105-106, 109
Company Name: SKW Trostberg AG

RECOMMENDATION:

Submitted as a new active ingredient Section 3 registration request.

The data are adequate to make a complete toxicological evaluation of the subject product.

Proposed product label (submitted on 10/28/93 by Siemer and Associates, Inc.) identifies all potential acute hazards indicated by the data reviewed.

Registration is recommended.

_____ _____
Peter Leung, Ph.D. Date
Staff Toxicologist

_____ _____
Gary Patterson, Ph.D. Date
Senior Toxicologist

_____ _____
Joyce Gee, Ph.D. Date
Senior Toxicologist

TO: James Herota, Registration Specialist
Pesticide Registration Branch

FROM: Medical Toxicology Branch 9/8/93 (revised)
12/28/87 (original)

DATA PACKAGE SUMMARY AND RECOMMENDATION SHEET - NEW ACTIVE INGREDIENT

Active Ingredient: Hydrogen Cyanamide
Formulated Product Name: Dormex
Formulation (excluding inerts): 51% hydrogen cyanamide, 49% inerts
Chemical Code #: 2238 **ID #:** 135613N
EPA Reg #: **SB 950 #:** New A.I.
Document #'s: 50660-003, 004, 012, 020, 035-039, 040, 043-044, 048, 071-072, 075-077, 105-106, 109
Company Name: SKW Trostberg AG

SUMMARY: ("DPR One-Liners" from each study worksheet, significant information not mentioned in worksheets, other pertinent information for ongoing review or registration.)

ACUTE STUDIES - Technical

The manufactured product is approximately 50% hydrogen cyanamide so the aqueous solution is equivalent to technical material.

	Toxicity Category
Acute Oral Toxicity, LD_{50}	II
Acute Dermal Toxicity, LD_{50}	II
Acute Inhalation Toxicity, LC_{50}	not required at this time
Primary Eye Irritation	III
Primary Skin Irritation	IV

Acute Oral Toxicity

50660-003; 34911; Acute Oral Toxicity Study; 811; Rat; Central Institute for Nutrition and Food Research, Zeist, The Netherlands; No study no.; 2/7/73; Cyanamid L 500; 5 animals/sex/group; Dose: 0.20, 0.25, 0.30, 0.35 ml/kg; Mortality: 0.20 (M:1/5, F:0/5), 0.25 (M:4/5, F:0/5), 0.30 (M:5/5, F:2/5), 0.35 (M:5/5, F:1/5); Observations: convulsions; Necropsy: no treatment-related lesions in the survivors; LD50 (M/F): 0.285 ml/kg; Toxicity Category II; Study **acceptable**. (Moore, 8/2/93)

Acute Dermal Toxicity

50660-003; 34910; Acute Dermal Toxicity Study; 812; Rabbit; Central Institute for Nutrition and Food Research, Zeist, The Netherlands; No study no.; 6/19/73; Cyanamid L 500; 2 animals/sex/group; Doses: 0, 2.0, 4.0, 6.0 ml/kg, 24 hour exposure; Mortality: 0 (M/F:0/2), 2.0 (M/F:0/2), 4.0 (M:1/2, F:0/2), 6.0 (M/F:2/2); Observations: signs of apathy, dilation of pupils, skin irritation;

Necropsy: swollen livers, hemorrhagic erosions in the stomach; Histopathology: acanthosis, parakeratosis, hyperkeratosis, edema in the skin, enlarged periportal hepatocytes, atrophy of splenic white pulp, hemorrhagic erosions in the stomach; LD50 can not be determined; Toxicity Category can not be determined;Study **unacceptable, not upgradeable** (number of animals/sex/group less than the recommended number of 5). (Moore, 8/2/93).

50660-035; 85349; Acute Dermal Toxicity Study; 812; Rabbit; Hazleton Laboratories America, Inc., Vienna, VA; Study No. 2319-122; 2/9/88; aqueous hydrogen cyanamide (50% w/w); 5 animals/sex/group; Doses: 1.0, 2.5, 4.0 ml/kg, 24 hour exposure; Mortality: 1.0 (M:0/5, F:1/5, 2.5 (M/F:5/5), 4.0 (M/F:5/5); Observations: prostrate, depressed, tremors, ataxia; Necropsy: dark red areas in lungs; LD50 (95% confidence limits) M=1.7 (1.1 to 2.7) ml/kg, F=1.4 (0.9 to 2.2) ml/kg; Toxicity Category II; Study **acceptable**. (Moore, 8/2/93)

Acute Inhalation Toxicity

50660-003; 34909; Acute Inhalation Toxicity Study; 813; Rat; Central Institute for Nutrition and Food Research, Zeist, The Netherlands; No study no.; 5/22/73; SKW Cyanamid L 500; 5 animals/sex; reported exposure concentration (analytical) 2.0 gm/m^3; reported particle size-99% less than or equal to 1.5 mm; No mortality; Observations: rapid, shallow respiration during exposure; Necropsy: no treatment-related lesions reported; reported LC50 (M/F) > 2.0 gm/m^3; Summary Study (information regarding the analytical data and calculations used to determine the exposure concentration, the data collected to determine the particle size distribution, the observation records for the study animals, and the necropsy records for the animals were not included in the report). (Moore, 8/2/93)

Primary Eye Irritation

50660-012; 42360; Primary Eye Irritation Study; 814; Rabbit; Central Institute for Nutrition and Food Research, Zeist, The Netherlands; No study no.; 6/10/74; SKW-Cyanamid L 500; 6 animals; Dose: 0.1 ml; 6 animals; Observations: corneal opacity, grade 1 or 2 in all animals at 24 and 48 hours, gradually diminishing and clearing by 7 days; iritis, grade 1 in all animals at 24 and 48 hours, diminishing to grade 1 in 4/6 at 72 hours and clearing by 7 days; conjunctival irritation-redness, grade 2 in all animals at 24 and 48 hours, diminishing to grade 1 or 2 in all animals at 72 hours and grade 1 in all animals at 7 days, chemosis-grade 2 or 3 in all animals at 24 and 48 hours, diminishing to grade 1 in 5/6 animals at 7 days; Toxicity Category III; Study **acceptable**. (Moore, 7/30/93)

Primary Skin Irritation

50660-012; 42359; Primary Dermal Irritation Study; 815; Rabbit; Central Institute for Nutrition and Food Research, Zeist, The Netherlands; No study no.; No study date; SKW-Cyanamide L 500; 6 animals; Dose: 0.5 ml, 4 hour exposure; Observations: erythema-grade 1 to 4 for all animals at 4 hours postdose, grade 2 or 4 for all animals at 52 hours postdose, edema-grade 2 or 3 for all animals at 4 hours postdose, grade 1 or 2 for all animals postdose; Toxicity Category can not be determined; Study **unacceptable, not upgradeable** (study terminated prior to reversal of irritation signs). (Moore, 7/30/93)

50660-071; 116268; Primary Dermal Irritation Study; 815; Rabbit; Huntingdon

Research Centre Ltd., Huntingdon, Cambridgeshire, England; Lab. No. 891330D/STB 4/SE; 12/22/89; Aqueous Hydrogen Cyanamide 49% w/w; 6 animals; Dose: 0.5 ml, 4 hour exposure; Observations: erythema-grade 1 or 2 in all animals at 4 hours post-dosing, diminishing to grade 1 in 2 animals at 24 hours, clearing by 48 hours, edema-grade 1 in 5/6 animals at 4 hours, clearing by 24 hours; Toxicity Category IV; Study **acceptable**. (Moore, 7/30/93)

SUBCHRONIC STUDIES

Oral

072; 116269; 28-Day Repeated Dose Oral Toxicity Study with Aqueous Hydrogen Cyanamide in Rats; Author: Osheroff, M.R.; 821; Rat; Hazleton Laboratories America, Inc., Rockville, MD; Study No. 2319-123; 9/9/88; Aqueous Hydrogen Cyanamide (50% A.I. (w/w)); 5 animals/sex/group; Doses: 0 (I), 5 (II), 10 (III), 20 (IV), 40 (V) mg/kg/day a.i., by gavage; Mortality: I (M/F:0/5), II (M/F:0/5), III (M/F:0/5), IV (M:0/5;F:1/5), V (M/F:0/5); Clinical Observations: rough haircoat (V); decreased body weight (IV M, V M,F); food consumption decreased (IV, V M); Hematology: decrease in rbc count (V M), hemoglobin (V M,F), hematocrit (V M,F), and MCH (V M); Clinical Chemistry: increase in BUN (V M), no apparent effect on parameters of thyroid function test; Gross Necropsy: no apparent treatment-related lesions, reduced absolute testes weight (IV, V); increased organ/body weight ratio for brain (V M), kidney (IV and V M,F), liver (IV M, V M,F) and thryroid (V M); Histopathology: thyroid-decreased colloid, hyperplasia of follicular cells, small, closely packed follicles, apparent dose-response; liver-bile duct hyperplasia, apparent dose-response; kidneys-mineralization in tubules (V M); **target tissues**: thyroid, liver; **possible adverse effect**: follicular cell hyperplasia in thyroid, bile duct hyperplasia in liver; NOEL < 5 mg/kg/day a.i. (based on histopathological changes in thyroid and bile duct hyperplasia in 5, 10, 20, and 40 mg/kg/day a.i. treated males); Study supplemental. (Moore, 7/30/93)

**** 036; 34903**; Sub-Chronic (90-Day) Oral Toxicity Study with Alzodef in Dogs (Authors: H.P. Til, *et. al.*); 821; Dog; Netherlands Organization for Applied Scientific Research, Division for Nutrition and Food Research TNO, Zeist, The Netherlands; Project ID# V 82.084/210694; 3/82; 4 animals/sex/dose; Dose: 0 (I), 0.6 (II), 2 (III), 6 (IV) mg/kg/day a.i., by gavage; No mortality; Clinical observations: no treatment-related signs, reduced body weight gain (IV F); Hematology: reduced hemoglobin, packed cell volume, rbc count, day 85 (IV M), increased % of monocytes (III F, IV M/F); Urinalysis: no treatment-related effects; Function Tests: no treatment-effect on liver or kidney, reduced T3 content, day 85 (IV M/F), reduced T4 content, day 85 (IV M); Clinical Chemistry: increased cholesterol level, days 44, 85 (IV F), reduced potassium level, days 44, 85 (IV M); Necropsy: reduced testes weight (IV M); Histopathology: reduced or absent spermatogenesis (II, III, IV); **target organ**: testes; **possible adverse effect**: reduced or absent spermatogenesis; NOEL can not be determined (reduced spermatogenesis in 0.6 mg/kg/day a.i. treatment group); Study **acceptable**. (Moore, 8/3/93)

003; 34904; Sub-Chronic (90-Day) Toxicity Study with Cyanamid L 500 in Albino Rats (Authors: H.P. Til, *et. al.*); 821; Rat; Central Institute for Nutrition and Food Research, Zeist, The Netherlands; Report No. R 4595; 1/24/75; Cyanamid L 500; 10 animals/sex/group; Doses: 0 (I), 20 (II), 60 III), 180 (IV) ppm; No mortality; Clinical Observations: no treatment-related signs, no treatment-related effects

upon body weights or food consumption; Hematology: slight increase in rbc count (IV M); Clinical Chemistry: no treatment-related effects observed; Urinalysis: no treatment-related effects observed; Necropsy: no treatment-related lesions, relative liver weight slightly increased (IV M), relative thymus weight slightly reduced (IV F); Histopathology: predominantly small follicular lumens without colloid in thyroid (III M, IV M,F); **target organ**: thyroid; **possible adverse effect**: increased number of small follicles, lined with cuboidal epithelial cells, disappearance of colloid in thyroid; nominal NOEL = 20 ppm; **unacceptable, not upgradeable** (no analysis of the test article in the diet was performed). (Moore, 8/4/93)

003; 34905; Range-Finding (28 Day) Toxicity Study with Cyanamid L 500 in Albino Rats (Authors: H.P. Til, *et. al.*); 821; Rat; Central Institute for Nutrition and Food Research, Zeist, The Netherlands; Report No. R 4387; 5/21/74; Cyanamid L 500; 10 animals/sex/group; Doses: 0 (I), 100 (II), 300 (III), 1000 (IV), 3000 (V) ppm; Mortality: 0 (M/F:0/10), 100 (M/F:0/10), 300 (M/F:0/10), 1000 (M/F:0/10), 3000 (M:0/10; F:1/10); Clinical Observations: emaciation, decreased activity, reduced body weight gain or body weight loss, reduced food consumption (III, IV, V); Hematology: reduced hemoglobin concentration (IV, V); Necropsy: grossly pale livers (V), increased relative organ weights for liver (IV, V) and kidney (V); Histopathology: hydropic liver cell degeneration, ballooning of liver cells with marked degree of bile duct proliferation (IV, V), slight to moderate enlargement of periportal hepatocytes, scattered liver cell necrosis, occasional hydropic degeneration of liver cells (II, III); **target organ**: liver; **possible adverse effect**: marked lesions in the liver; **NOEL**: < 100 ppm (based on treatment-related effects in the liver of the 100 ppm treatment group); **Study Supplemental**. (Moore, 8/4/93)

038; 85352; "6-Week Oral (Drinking Water) Palatability and Dose Range Finding Study in the Mouse" (author: Goodyer, M.J., Hazleton Laboratories Europe, Ltd., North Yorkshire, UK, lab. project ID # 5051-556/2, 5/86); aqueous hydrogen cyanamide (49% a.i.) administered in drinking water for 6 weeks; mean actual dosage level: male - 0, 26.0, 72.3, or 171.1 mg a.i./kg/day, female - 0, 37.4, 88.9 or 204.4 mg a.i./kg/day; 6 mice/sex/dose; all animals survived the 6-week treatment period; no treatment-related changes in clinical conditions or behavior; slight decrease in mean body weight in high dose males; water consumption for high dose males and females was markedly reduced throughout the treatment period; body weight was unaffected by treatment at the mid dose; however, water consumption at this dose level was slightly reduced; no abnormalities were seen at necropsy; Therefore the mid dose was selected as the high dose for the subsequently chronic study in mice; **supplemental**; (Leung, 8/5/93).

METABOLISM STUDIES

109; 125670; "Metabolism of [^{14}C]-Hydrogen Cyanamide in Rats (Preliminary and Definitive Phases" (author: Struble, C.B., Hazleton Wisconsin, Madison, WI, Lab. Project ID # HWI 6265-101, 8/19/93); Hydrogen cyanamide (radiolabeled: lot # 061H9212, 97% purity, 14.8 mCi/mmol; nonlabeled: lot # 111701, 99.7% purity); oral: single (1 or 20 mg/kg) or multiple (pretreated with 1 mg/kg/day for 14 days with nonlabeled hydrogen cyanamide followed by a final dose of radiolabeled hydrogen cyanamide), IV: single (1 mg/kg); 5 rats/sex/dose; radioactivity rapidly excreted, regardless of the route of administration or dose level; 67% to 92% excreted by all routes in the first 24 hours after administration; main route of excretion was via urine (66.9 - 97.7%); fecal excretion of radioactivity was 3 to 4 times higher after an IV dose (13.2 - 15%) compared with a similar oral dose (2.3 - 4.2%), suggesting that biliary excretion may play an important role after IV dosing; elimination of radioactivity as $^{14}CO_2$ reduced by 30% in animals repeatedly dosed with hydrogen cyanamide as compared with single oral dose; treatment at 20 mg/kg reduced the percent of radioactivity in expired air but the opposite result was observed for the percent of dose in the urine; no evidence of bioaccumulation after multiple dosing; major metabolic reaction was acetylation of the nitrogen; N-acetylcyanamide acounts for 58% to 74% of the radioactivity in urine and for over 80% in feces; high concentrations of radioactivity found in blood, liver and kidney; males were reported to have higher levels of radioactivity in liver, blood and kidney as compared to females; **acceptable**; (Leung, 9/1/93).

048; 92525; "Investigation of the Absorption, Metabolism, and Excretion of Hydrogen Cyanamide in Rat and Human" (authors: Gloxhuber, C. et. al., SKW Trostberg AG, Trostberg, Germany, 9/1/89); The major urinary metabolite of cyanamide in rat and man is N-acetylcyanamide. Rats treated orally with cyanamide (10 mg/kg) demonstrate that 51.5% of the dose was excreted in the urine as N-acetylcyanamide. Similarly, male human volunteers excreted 57.8% of the oral dose (20 mg/kg) in urine as N-acetylcyanamide. The same group of volunteers participated in a skin absorption with dermal application of 20 mg per person. A maximum of 3.5% of this dose was absorbed through the skin and excreted as N-acetylcyanamide in the urine. Additional findings from the literature indicate that cyanamide is metabolized in vitro to cyanide. However, according to experiments performed in vivo, no significant increases in cyanide concentrations in the blood and thiocyanate concentrations in the urine were detected after a 20 mg oral dose. **Supplemental**. (Leung, 8/10/93).

077; 116275; "Metabolism of Hydrogen Cyanamide" (author: Environ Corp., 9/5/91)); Hydrogen cyanamide is rapidly absorbed and eliminated following oral administration in rats (t1/2 = 27.2 minutes) and dogs (t1/2 = 62 minutes). In contrast, absorption through the skin is generally proportional to the dose and increases with the time of exposure. The major urinary metabolite after oral dosing in rat, dog, and man has been identified as N-acetylcyanamide and accounts for 45.6%, 87%, and 87%, of the administered dose, respectively. Blood samples of volunteers collected before and up to 48 hours after intake of hydrogen cyanamide did not show any significant differences in blood levels of cyanide or urinary levels of thiocyanate. Therefore, the possible metabolic pathway of hydrogen cyanamide to cyanide as found in vitro is irrelevant for man. **Supplemental data**. (Leung, 8/10/93).

SB950-MANDATED HEALTH EFFECTS STUDIES

Combined, Rat

not submitted and not required at this time

Chronic Toxicity, Rat

** 043, 105; 85432, 116545; "Chronic Toxicity Study in Rats with Aqueous Hydrogen Cyanamide" (author: Osheroff, M.R., Hazleton Laboratories America, Inc., Rockville, MD, HLA Study # 2319-125, progress report (7/28/89), final report (4/15/91)); 831; aqueous hydrogen cyanamide (50% w/w a.i.) administered by gavage to 20 Sprague-Dawley rats/sex/dose for 92 weeks; treatment initiated at 0, 2.5, 7.5, or 30 mg/kg/day a.i. was reduced at the 17th week to 0, 1, 2.5, and 7.5 mg/kg/day a.i., respectively, due to deterioration in general health; high dosed animals were noted to be "hunched" with tremors during weeks 9 - 17; decreases in body weight reported for mid and high dose animals at weeks 4 and 16 and for high dose animals at weeks 52 and 91; compound-related histomorphologic alterations consisted of reduced colloid, characterized by microfollicles, in the thyroid of mid- and high-dose males (7/20, 17/18, respectively vs 0/20 control) and high-dosed females (16/20 vs 3/20 control); reduced plasma levels of triiodothyronine (T3) in high dose males and females and in mid dose males at week 92; thryoxine (T4) was also reduced in high dose males at weeks 14 and 92, while thyrotropin was comparable between control and treated groups; NOEL (M/F) = 1 mg/kg/day a.i. (reduced body weight and changes in T3 and T4); **no adverse effects; acceptable**; (Aldous, 10/23/90, updated, Leung, 8/2/93).

Chronic Toxicity, Dog

**** 037; 85351;** "Chronic Toxicity Study in Dogs with Aqueous Hydrogen Cyanamide" (author: Osherhoff, M.R., Hazleton Laboratories America, Inc., Vienna, VA, HLA Study # 2319-121, 5/10/89); 831; aqueous hydrogen cyanamide (Lot # 07/07/87, 50% w/w) administered in distilled water to 4 dogs/sex/day for 52 weeks; treatment initiated at 0, 0.1, 0.5, or 2.5 mg a.i./kg/day was increased to 0, 0.2, 1.0, or 5.0 mg a.i./kg/day on week 2; no deaths were reported during the course of this study; salivation and tremors were noted in high dose animals and in one mid dose female; **possible adverse effect:** inflammation and decreased spermatogenic activity were noted in the testes of three high dose dogs; in one dog, inflammation was rather severe in both testes and aspermatogenesis was evident; histological sections of testes with epididymides revealed the presence of immature sperm forms and neutrophil infiltration in the epididymal ducts of one mid dose dog; significant reduction in thyroxin levels and concomitant signs of decreased metabolic rate in high dose dogs indicate hypothyroidism; thymic atrophy was noted in high dose males and one high dose female with demodicosis; NOEL (M/F) = 0.2 mg a.i./kg/day (clinical signs and histopathological findings); **acceptable**; (Aldous, 10/26/90; updated, Leung, 8/4/93).

Oncogenicity, Rat

50660-004; 34921; "Bioassay of Calcium Cyanamide for Possible Carcinogenicity" (NCI Frederick Cancer Research Center, DHEW Pub. 79-1710) Calcium cyanamid, 63% commercial formulation with 22% calcium oxide and 12% free carbon, fed in the diet to F344 rats, 20/sex in controls, diets replenished at least 3 times per week;

50/sex in test groups, two doses only; dietary levels up to 400 ppm; no toxicity or carcinogenicity observed; incomplete and **unacceptable** report (no diet analysis, no individual data supplied, doses too low, 2 studies in same room). **Not upgradable;** (Berliner and Christopher, 10/28/85.

Oncogenicity, mouse

**** 051, 106; 95230, 116548;** "Hydrogen Cyanamide: Up to 104 Week Oral (Drinking Water) Carcinogenicity Study in the Mouse" (Goodyer, M.J., Hazleton UK, North Yorkshire, UK, Lab. Study # 6001-556/3, final report: 5/90, amended final report: 10/1991); 832; Hydrogen Cyanamide (49.9% purity, w/w) administered in drinking water to 60 mice/sex/dose for 104 weeks; mean actual dose levels: males - 0, 10.7, 29.5, or 75.6 mg a.i./kg/day, females - 0, 13.7, 35.2, or 101 mg a.i./kg/day; dose-related increase in the incidence of chronic cystitis of the urinary bladder was reported for mid and high dose animals; evidence of kidney degeneration as characterized by atrophic/basophilic tubules, fibrosis/scarring and vacuolar degeneration was detected in high dose animals; **possible adverse effect:** slight increased incidence of granulosa theca tumors in ovaries in high dose females would have been significant if one of the three granulosa theca tumors in the control group, which was diagnosed as "questionable" due to tissue necrosis, was not included in analysis; comparison of the incidence of ovarian granulosa-theca tumors in the present study with the average incidence obtained from historical control data from 1985 to 1990 revealed significant increases in mid and high dose females; NOEL (M) = 10.7 mg/kg/day a.i., (F) = 13.7 mg/kg/day a.i.(based on chronic cystitis of the urinary bladder and ovarian granulosa-theca tumors); **acceptable**; (Aldous, 10/26/90; updated, Leung, 8/3/93).

50660-004; 34921; "Bioassay of Calcium Cyanamide for Possible Carcinogenicity" (NCI Frederick Cancer Research Center, Pub. 79-1719) Calcium cyanamide, 63%, calcium oxide 22%, free carbon, 12%; fed in the diet to B6C3F1 mice at 0, 500 or 1000 ppm for 100 weeks; Inadequate. Unacceptable (no analyses of diets, no individual data, no body weights, seven other studies in same animal room). Possible oncogenic effect (hemangiosarcomas in males). Considered **supplemental data** for hydrogen cyanamide; (Christopher, 10/25/85).

Reproduction, Rat

019 63684 "Oral Two-Generation Reproduction Study with An Aqueous Cyanamide Solution (Content 49% W/W) in Rats." (Netherlands Organization for Applied Scientific Research, 10/86, 84-1475) Aqueous cyanamide (49% W/W, no further identification of other 51% or whether calcium or hydrogen form at start); fed in the diet at 0, 20, 60 or 180 ppm to 24/sex/group, two generations, two litters per generation; testicular changes described as partial or complete impairment of spermatogenesis occurred in all groups but particularly in the high dose, F1 males but fertility was not obviously impaired; body weight gains were decreased in both males and females at 180 ppm; no effect was reported on other reproductive parameters; NOEL (males) < 20 ppm (testicular changes), females = 60 ppm (body weight), reproductive NOEL $\geq$ 180 (no consistent effects on litter/pup data). **Unacceptable**, incomplete - no individual data, inadequate description of the test material, mean value only for 10 analyses of the diet in which there was a problem with stability. **Upgradeable**; (Gee, 12/21/87).

** 075; 116273; "Two-Generation Reproduction Study in Rats with Aqueous Hydrogen Cyanamide (50% w/w)", (Author: S.L. Morseth; Hazleton Laboratories America Inc., Vienna,VA; Report No. 2319-126; 4/19/90; Dormex (50% w/w Hydrogen Cyanamide); 0, 1.25, 3.75, 15.0 mg/kg/day of a.i. oral gavage; 26 Charles River rats/sex/dose level both F_0, F_1; 2 generations, 1 mating/generation; Adult Effects: no dose related mortalities, 2 animals died due to gavage error; initial high dose levels(7.25 and 30 mg/kg/day of a.i.) resulted in weight loss, decreased food consumption and rough haircoats; dose levels were reduced at week 12, 2 weeks before mating and high dosed rats gained weight but showed reduced fertility and gestation indices; Offspring Effects: high dose group showed decreased day 4 mean litter size and body weights(day 7-day 21), mean pup weight(day 7-day 21) reduced at 3.75 mg/kg level; reduced day 4 viability observed in F_0 and F_1 generations at all treatment levels, however, these observations can only be attributed to treatment and not specific dose levels because of dose level changes; F_1 animals at all treatment levels had decreased growth after weaning and gavage dosing began; lower F_2 neonatal survival in treated groups was attributed to variability not treatment results, pups that were weak, thin, dehydrated, and had dry skin were noted not to occur in a dose-related pattern; gross necropsy and histomorphologic findings in adults and offspring were not considered treatment related; **no adverse effects indicated;** Adult NOEL: 1.25 mg/kg/day a.i. (based on reduced body weight); Developmental NOEL:1.25 mg/kg/day a.i. (based on reduced body weight); **Acceptable.** (Miller, 8/3/93)

Teratology, Rat

** 030, 040; 70870, 85355; Rat Teratology Study with Aqueous Hydrogen Cyanamide (Authors: S.L. Morseth, *et. al.*); 833; Rat; Hazleton Laboratories America, Inc., Vienna, VA; Study No. 2319-124; 5/2/89; Aqueous Hydrogen Cyanamide (50% (w/w) hydrogen cyanamide); 25 animals/group; Dose: 0 (I), 5 (II), 15 (III), 45 (IV) mg/kg/day a.i., gavage, from 6 through day 15 of gestation; Maternal: No mortality; Clinical Observations-hypoactivity (III, IV), reduced body weights and food consumption from gestation day 8 through 20 (III, IV), mean gravid uterine weight reduced (IV); Necropsy-no treatment-related lesions, no apparent treatment-related effect on post-implantation loss; Developmental: mean fetal weight reduced (IV), no apparent treatment-related effect on external malformations; soft tissue malformations-diaphragmatic hernia (IV); increased incidence of certain skeletal variations (IV), no apparent-treatment related effect on skeletal malformations; no adverse effect; Maternal NOEL = 5 mg/kg/day a.i.(based on the reduced food consumption and body weight gain exhibited by the females in the 15 mg/kg/day treatment group); Developmental NOEL = 15 mg/kg/day a.i.(based on reduced fetal weight and incidence of soft tissue malformations and increased number of particular skeletal variations observed in the 45 mg/kg/day a.i. treatment group); initially unacceptable but possibly upgradeable with submission of finalized report with GLP compliance statement, purity and stability of test material; **no adverse effects**; subsequently reviewed as **acceptable**; (Rinkus, 11/10/88; upgraded, Moore, 8/5/93)

Teratology, Rabbit

**50660-004, 039, 076; 34919, 85354, 116274; "Oral Embryotoxicity/Teratogenicity Study with an Aqueous Cyanamid Solution (49%) in New Zealand White Rabbits."(Author: Koeter, H.B.W.M., and van Marwijk, M.W.; TNO-CIVO Division for Nutrition and Food Research, Netherlands; Report No. 84.444/240171; 11/84); Dormex (Batch No. 06/04/91; 49% Hydrogen Cyanamide); 0, 4, 12, 36 mg/kg/day (0, 2, 6, 18 mg/kg/day a.i. respectively) oral gavage; New Zealand White rabbits; at 36 mg/kg:

demonstrated very slight maternal toxicity (decreased b.w. gain) and developmental toxicity (increased resorptions, deaths, anomalies and variants, decreased fetal weight); 12 mg/kg dose: increased anomalies and variants; Maternal NOEL = 12 mg/kg/day (6 mg/kg a.i.) (based on decreased body weight gain); Developmental NOEL = 4 mg/kg/day (2 mg/kg a.i.) (based on increased retinal folds); **no adverse effects;** initially reviewed as unacceptable but possibly upgradeable with submission of additional data; requested information submitted and subsequently reviewed as **Acceptable.** (Parker, 10/23/85; upgraded, Miller, 8/6/93)

Mutagenicity, gene

50660-004; 34917; "Evaluation of Two Products CCA and CA in the Salmonella/ microsome Mutagenicity Test." (author: Willems, M.I., Central Institute for Nutrition and Food Research, The Netherlands, Report # R 5707, 6/78) Salmonella microsome mutagenicity for "CCA" and "CA", tested at 0 to 1000 ug/plate, in triplicate, with and without activation by aroclor 1254-induced rat liver S9 fraction; CCA is calcium cyanamide and CA is cyanamide; **Unacceptable and not upgradeable** (because high concentration not justified, no repeat trial to confirm negative result, no individual plate counts). **no adverse effects indicated**; No increase in reversion reported. (Berliner and Gee, 10/22/85; updated, Leung, 8/5/93).

** 50660-020; 63691; "Mutagenicity Test on Hydrogen Cyanamide in the Ames Salmonella/Microsome Reverse Mutation Assay." (author: Jagannath, D.R., Hazleton Laboratories America, MD, HLA 9583-0-401, 10/21/87); Hydrogen cyanamide (53%, w/v in aqueous solution, lot 02/10/86); Salmonella strains TA1535, TA1537, TA1538, TA98 and TA100, two trials in triplicate, with and without activation by aroclor 1254-induced rat liver S9 fraction; concentrations of 0.02, 0.04, 0.08, 0.17, 0.42, 0.84, 1.69 and 2.54 mg hydrogen cyanamide per plate based on analyzed amount (0.10 to 15 ul); **no adverse effects**; no increase in reversion rate reported in either trial with decrease in reversion rate at the highest concentration as evidence of the adequacy of the high concentration; **Acceptable**. (Gee, 12/21/87; updated, Leung, 8/5/93).

Mutagenicity, chromosome

50660-004; 34914; "Evaluation of "Kalkstickstoff and "Thioharnstoff" in the Micronucleus Test" (author: Willems, M.I., Central Institute for Nutrition and Food Research, The Netherlands, Report # R 6012, 2/79); Kalkstickstoff (calcium cyanamide, calcium oxide and free carbon with 23% N - 50660-007) Tested in Wistar rats by oral gavage at 0, 3.06 and 7.0 %, 5/sex/group, 2 dosings, sacrificed 6 hours after the second dosing; 400 cells/slide with 5 slides per animal; trenimon as positive control; **No adverse effects indicated; Unacceptable** (no toxicity reported from high dose, single sacrifice time.) **Not upgradable.** (Berliner and Gee, 10/22/85; updated, Leung, 8/5/93).

50660-004; 34916; "An Investigation into the Sister Chromatid Exchange Induction in Chinese Hamster Ovary Cells by a Sample of "Kalkstickstoff." (author: de Raat, W.K., Netherlands Organization for Applied Scientific Research, Report # CL/78/120, 2/2/79) Calcium cyanamide 40-60%, calcium oxide and free carbon (Kalkstickstoff); Chinese hamster ovary cells tested for 1 hour with 0, 10, 50, 250 or 500 ug/ml; 500 ug/ml equivalent to 330 ug/ml calcium cyanamide and was not completely soluble; scored 20 metaphases per slide; **No adverse effects indicated;**

Unacceptable (inadequate number of metaphases scored, single replicate per concentration, exposure of 1 hour not justified), **not upgradable**. (Berliner and Gee, 10/22/85; updated, Leung, 8/5/93).

**** 50660-020; 63690**; "Mutagenicity Test on Hydrogen Cyanamide in an in vitro Cytogenetic Assay Measuring Chromosomal Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells." (author: Ivett, J.L., Hazleton Laboratories America, Inc., Kensington, MD, HLA 9583-0-437, 10/21/87.) Hydrogen cyanamide, 53% w/v aqueous solution, lot 02/10/86, liquid with sp. gravity of 1.06; CHO cells exposed in vitro without activation at 0, 42.4, 56.5, 141 or 283 ug/ml for 17.5 hours plus 2.5 hours = 20 hour harvest; with rat liver activation at 0, 438, 875 or 1310 ug/ml, 2 hour treatment and harvest at 20 hours or 0, 321 or 428 ug/ml, total of 10 hours to harvest; **possible adverse effects:** increase in aberrations with and without activation; **acceptable;** (Gee, 12/21/87; updated, Leung, 8/5/93)..

** 50660-020; 63689; "Mutagenicity Test on Hydrogen Cyanamide in the in vivo Mouse Micronucleus Assay." (author: Ivett, J.L., Hazleton Laboratories America, Inc., Kensington, MD, HLA Study # 10052-0-455, 10/21/87); Hydrogen cyanamide, 53% w/v aqueous solution, lot 7/07/87, liquid; given by oral gavage to 5/sex/test, ICR mice; actual doses from analysis at 0, 31.4, 157.4 or 330.5 mg/kg in water; 2 males in high dose group died within 24 hours; sacrifices at 24, 48 and 72 hours; no positive or negative controls at 48 and 72 hours; **no adverse effect;** no effect on micronuclei formation; **acceptable;** (Gee, 12/21/87; updated, Leung, 8/5/93).

Mutagenicity, DNA/other

** 50660-020; 63688; "Mutagenicity Test on Hydrogen Cyanamide in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay." (author: Cifone, M.A., Hazleton Laboratories America, Kensington, MD, HLA #. 9583-0-447, 10/21/87.) Hydrogen cyanamide, 53% w/v in aqueous solution; male rat hepatocytes tested at 0, 6.0, 11.9, 23.8, 47.6, 71.4, 95.2, 143 or 190 ug/ml actual content from analysis; triplicate coverslips, scored a total of 150 nuclei per concentration; **no adverse effect**; negative for unscheduled DNA synthesis; **acceptable**. (Gee, 12/21/87; updated, Leung, 8/5/93).

Literature review

50660-020 63686 "Potential Health Hazards of Dormex (Hydrogen Cyanamide) Exposure: A Literature Review (Draft)." (Authors: Environ's Staff; Environ Corporation, 7/24/87) Review discusses 2 reports on the incidence of cancer in workers in the manufacture of calcium carbimide = cyanamide in Norway and Germany. No cancer excess was noted. It also discussed treatment of alcoholics with calcium cyanamide with changes in the liver ("ground glass hepatocytes") the only finding other than intended effects of nausea, anorexia, flushing, etc. **supplemental**; (Gee, 12/21/87; updated, Leung, 8/5/93).

044; 87935; "Cyanamide-Induced Liver Cell Injury Experimental Study in the Rat" (author: Vazquez, J.J. and Guillen, F.J., Laboratory Investigation, 50: 385 - 393, 1984); Cyanamide prepared in saline and administered daily intraperitoneally for 27 weeks at 0, 8, or 16 mg/kg/day; 5-8 Wistar male rats/ dose; inclusion bodies, containing large amounts of glycogen, lipid droplets and secondary lysosomes were detected at week 13 of cyanamide treatment; initially hepatocytes bearing inclusion bodies are located predominantly at the periportal areas but moving toward the center of the lobule; prior to the detection of inclusion bodies, cyanamide treatment produced cytoplasmic homogenous areas consisting of glycogen depositions and smooth endoplasmic reticulum tubules; **supplemental**; (Leung, 8/6/93).

044; 87936; "Lack of Heptotoxicity after Long-Term Administration of Cyanamide in Rats: A Histological and Biochemical Study" (authors: Obach, R., Acta Pharmacol. et Toxicol. 57: 279-284, 1985); Cyanamide (Batches S-13 and T-14) prepared daily by dilution in distilled water were administered orally (0, 2, 7, or 25 mg/kg/day for 6 months, 20 Sprague-Dawley rats/sex/dose) or intraperitoneally (0, 8, or 16 mg/kg/day for 12 months, 40 male Wistar and 40 male Sprague Dawley rats/dose); no significant histological changes were observed in the liver after oral or intraperitoneal administration; specifically no inclusion bodies in any cyanamide-treated rats were detected; however, Sprague-Dawley rats treated orally for 6 months showed elevated levels of alkaline phosphatase, bilirubin, and alanine phosphatase at the high dose, whereas only bilirubin was increased in male Wistar rats treated intraperitoneally at 16 mg/kg/day for 12 months; **supplemental**; (Leung, 8/6/93).

CONCLUSIONS: Do data support registration?
Toxicity data to support Dormex have been submitted for Section 3 Registration to promote uniform budbraking in grapes after pruning.

All required acute toxicity studies conducted with hydrogen cyanamide are acceptable. An acceptable acute inhalation toxicity study is not required at this time because the product is applied as a coarse spray.

The subchronic oral toxicity study in dog is acceptable whereas the other study in albino rats is unacceptable. Testes and the thyroids have been identified as target organs in each respective study.

The animal metabolism study is acceptable.

The chronic toxicity study with rats is acceptable. Histopathological examinations of the thyroids from mid and high dose males revealed alterations consisting of reduced colloid as characterized by microfollicles. In addition, plasma levels of T3 were reduced in high dose animals and in mid dose males. However, thyrotropin was comparable between control and treated groups. but does not fulfill the current data requirements for an acceptable combined chronic/oncogenicity study. However, the duration of the study and the survival rate at the end of 18 months approaches that for a combined chronic/oncogenicity study. In addition, no tumors were detected in the present study. The bioassay of calcium cyanamide in rats did not show any evidence of carcinogenicity. Furthermore, no clear indication of mutagenicity was reported. Therefore, an acceptable oncogenicity study in rats is not required at this time.

The chronic toxicity study with dogs is acceptable and possible adverse effects consisting of testicular degeneration and hypothyroidism were identified.

The oncogenicity study in rats is unacceptable but the other study conducted with mice is acceptable with possible adverse effect. In the latter study, a slight increased incidence of granulosa theca tumors in ovaries in high dose females would have been significant if one of the three granulosa theca tumors in the control group, which was diagnosed as "questionable" due to tissue necrosis, was not included in analysis. Comparison of the incidence of ovarian granulosa-theca tumors in the present study with the average incidence obtained from historical control data from 1985 to 1990 revealed significant increases in mid and high dose females.

One of the two rat reproduction study is acceptable.

Acceptable teratology studies performed in rats and rabbits have been submitted.

Studies submitted to fulfill the data requirements for gene mutation, structural chromosomal aberration and other genotoxic effects categories are acceptable.

RECOMMENDATIONS: What type of registration action is being considered? In the case of ongoing registration, register or do not register? What other specific studies or data are requested?

Submitted as a new active ingredient Section 3 registration request.

The data are adequate to make a complete toxicological evaluation of the subject product.

Proposed product label (submitted on 10/28/93 by Siemer and Associates, Inc.) identifies all potential acute hazards indicated by the data reviewed.

Registration is recommended.

_____ _____
Peter Leung, Ph.D. Date
Staff Toxicologist

_____ _____
Gary Patterson, Ph.D. Date
Senior Toxicologist

_____ _____
Joyce Gee, Ph.D. Date
Senior Toxicologist

APPENDIX B

Calculation of Oncogenic Potency Factor

DATE: 08/10/1993 TIME: 06:32:15

GLOBAL 86 (MAY 1986)

BY RICHARD B. HOWE AND CYNTHIA VAN LANDINGHAM

CLEMENT ASSOCIATES
1201 GAINES STREET
RUSTON, LA 71270
(318) 255-4800

Cyanamide; F mice oral; granulosa theca

POLYNOMIAL DEGREE SELECTED BY PROGRAM, (POLY-DEGREE=0)
MONTE CARLO TEST USED IN SELECTION

GROUP	DOSE	#RESPONSES OBSERVED/#ANIMALS	#RESPONSES PREDICTED
1	.000000	2/ 59	1.59
2	13.7000	1/ 59	2.61
3	35.2000	6/ 60	4.24
4	101.000	8/ 58	8.55

CHI-SQUARE GOODNESS OF FIT STATISTIC IS 1.9708

P-VALUE FOR THE MONTE CARLO TEST IS .3400000000

FORM OF PROBABILITY FUNCTION:
P(DOSE) = 1 - exp(-Q0 - Q1 * D - Q2 * D^2)

MAXIMUM LIKELIHOOD ESTIMATES OF DOSE COEFFICIENTS

Q(0) = 2.729800324610E-02
Q(1) = 1.308378886321E-03
Q(2) = .000000000000

MAXIMUM VALUE OF THE LOG-LIKELIHOOD IS -57.6713212290

CALCULATIONS ARE BASED UPON EXTRA RISK
LINEARIZED MULTISTAGE CONFIDENCE LIMITS
****

RISK	MLE DOSE	LOWER BOUND ON DOSE	UPPER BOUND ON RISK	CONFIDENCE LIMIT SIZE
.10000	80.528	51.357	.15228	90.0
		46.187	.16781	95.0
		42.344	.18157	97.5
		38.473	.19790	99.0
1.00000E-02	7.6815	4.8989	1.56354E-02	90.0
		4.4057	1.73704E-02	95.0
		4.0392	1.89316E-02	97.5
		3.6700	2.08163E-02	99.0
1.00000E-03	.76469	.48768	1.56756E-03	90.0
		.43859	1.74288E-03	95.0
		.40210	1.90088E-03	97.5
		.36534	2.09192E-03	99.0
1.00000E-04	7.64343E-02	4.87462E-02	1.56796E-04	90.0
		4.38389E-02	1.74346E-04	95.0
		4.01918E-02	1.90165E-04	97.5
		3.65179E-02	2.09295E-04	99.0
1.00000E-05	7.64308E-03	4.87440E-03	1.56800E-05	90.0
		4.38369E-03	1.74352E-05	95.0
		4.01900E-03	1.90173E-05	97.5
		3.65162E-03	2.09305E-05	99.0
1.00000E-06	7.64305E-04	4.87438E-04	1.56800E-06	90.0
		4.38367E-04	1.74353E-06	95.0
		4.01898E-04	1.90174E-06	97.5
		3.65161E-04	2.09306E-06	99.0
1.00000E-07	7.64305E-05	4.87438E-05	1.56800E-07	90.0
		4.38367E-05	1.74353E-07	95.0
		4.01898E-05	1.90174E-07	97.5
		3.65161E-05	2.09306E-07	99.0
1.00000E-08	7.64304E-06	4.87438E-06	1.56800E-08	90.0
		4.38367E-06	1.74353E-08	95.0
		4.01898E-06	1.90174E-08	97.5
		3.65161E-06	2.09306E-08	99.0

END OF LINEARIZED MULTISTAGE CONFIDENCE LIMITS

****

GLOBAL 86 LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK
****

RISK	MLE DOSE	LOWER BOUND ON DOSE	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
1.00000E-05	7.64308E-03	4.38369E-03	95.0%	Q(0) = 1.76491E-02 Q(1) = 2.28119E-03 Q(2) = .00000
1.00000E-06	7.64305E-04	4.38367E-04	95.0%	Q(0) = 1.76491E-02 Q(1) = 2.28119E-03 Q(2) = .00000

GLOBAL 86 UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE
****

DOSE	MLE RISK	UPPER BOUND ON RISK	CONFIDENCE LIMIT SIZE	COEFFICIENTS FOR CONFIDENCE LIMIT
3.0000	3.91744E-03	6.82022E-03	95.0%	Q(0) = 1.76491E-02 Q(1) = 2.28119E-03 Q(2) = .00000
5.00000E-08	6.54189E-11	1.14060E-10	95.0%	Q(0) = 1.76491E-02 Q(1) = 2.28119E-03 Q(2) = .00000

CALCULATION OF POTENCY FOR HUMANS:

assuming Human BWt = 54.8 kg; mouse BWt = 0.03 kg

MLE Potency$_{hum}$: $1.3E\text{-}3 \times (54.8/0.03)^{1/4} = 8.5 \times 10^{-3}$

UB Potency$_{hum}$: $2.3E\text{-}3 \times (54.8/0.03)^{1/4} = 1.5 \times 10^{-2}$

ESTIMATION OF EXPOSURE OF PERSONS IN CALIFORNIA TO PESTICIDE PRODUCTS THAT CONTAIN HYDROGEN CYANAMIDE

BY

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HS-1685, November 30, 1993

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ABSTRACT

Hydrogen cyanamide is currently under review in California for a FIFRA Section 3 registration. Hydrogen cyanamide is applied to grape vines during dormancy to promote uniform bud break. It has been used in California under FIFRA Section 18 Emergency Exemption for three of the last six years. There are no reports of illness or injury in California involving this chemical . Dermal absorption of hydrogen cyanamide was 11.2 percent in laboratory rats. Following oral administration, hydrogen cyanamide was rapidly eliminated in tested animals and humans, mostly in urine. N-acetylcyanamide was the primary metabolite in urine. The estimates of exposure of workers involved in hydrogen cyanamide handling are based on three biological monitoring studies during actual work practices. The absorbed daily dosages for a mixer/loader and an applicator were 11.6 and 5.3 ug/kg/day, respectively. Seasonal average daily dosages (SADD) were calculated based on 12 and 26 workdays in a season for a mixer/loader and an applicator, respectively. The SADD was 2.3 ug/kg/day for both mixer/loaders and applicators

This report was prepared as a part of the Department's risk characterization document for hydrogen cyanamide because of possible adverse effects identified in subchronic and chronic toxicity studies in laboratory animals.

HUMAN PESTICIDE EXPOSURE ASSESSMENT

California Environmental Protection Agency
Department of Pesticide Regulation
Worker Health and Safety Branch

HYDROGEN CYANAMIDE

Novermber 30, 1993

Introduction

Hydrogen cyanamide has been used as a desert grape growth regulator in California for three of the last six years under a Section 18 Emergency Exemption of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Currently, hydrogen cyanamide is under review in California for registration under Section 3 of the FIFRA. The Department of Pesticide Regulation (DPR) is preparing a risk assessment document for hydrogen cyanamide because of possible adverse effects identified in subchronic and chronic toxicity studies in laboratory animals. Human exposure assessment is essential information for the risk assessment of pesticides. This human exposure assessment document will be an integral part of the Risk Characterization Document of the DPR for hydrogen cyanamide. It will also serve as a basis for developing mitigation strategies if exposure to hydrogen cyanamide is found to cause excessive theoretical risk.

Chemical and Physical Properties

Hydrogen cyanamide (CAS # 420-04-2) has a molecular weight of 42.04 g/mole. Its empirical formula is CH_2N_2. It melts at 45 to 46^{o}C. It is a hygroscopic compound with an octanol/water partition coefficient of -0.82 at 20^{o}C. Hydrogen cyanamide is soluble in water (100 g/100 mL at 43^{o}C) and alcohols. The vapor pressure is 5×10^{-3} Pa at 20^{o}C.

U.S. EPA and California Status

Currently, hydrogen cyanamide is under concurrent reviews in the DPR and the U.S. EPA for registration under Section 3 of the FIFRA.

Formulations

Dormex™ is the formulated product of hydrogen cyanamide. It is a liquid concentrate formulation containing 51 percent active ingredient (a.i.) or 4.3 pounds (lb.) a.i. per gallon.

Usage

Hydrogen cyanamide is applied once a year to dormant grape vines in the winter to promote uniform bud break in the spring. A four percent (v/v) dilution of Dormex™ and 0.25 to 0.50 percent non-ionic surfactant is applied as a coarse spray at a rate of 50 to 100 gallons of spray per acre of grape vines. This is equivalent to a

maximum of 17.2 lb. of hydrogen cyanamide per acre. The use season is between December first and January 31.

Label Precautions

Since hydrogen cyanamide is under concurrent review both in California and the U.S. EPA, no federally approved label is available at this time. The information on the proposed label indicates that the formulated product is a federally restricted use pesticide. It is a liquid toxicity category one material bearing the signal word "Danger". California Code of Regulations (section 6746) require closed system mixing and loading of liquid toxicity category one pesticides. The proposed product label provides precautionary statements to the user regarding the hazards of ingestion, inhalation, and skin and eye contact. The label requires the mixer/loaders to wear chemical resistant protective clothing, chemical resistant gloves, chemical resistant boots, NIOSH/MSHA-approved respirator, goggles, and apron. Applicators must use closed cabs with functioning activated charcoal air-intake filters and positive-pressure ventilation. The operating pressure of the spray rig must be no more than 40 psi with the use of low pressure nozzles. Applicators must wear chemical resistant protective clothing, and chemical resistant boots. Gloves, goggles, and a respirator must be worn when exiting the enclosed cab. Supervisors involved in handling must wear the same protective clothing and equipment as required for mixer/loaders, except an apron. A 100-yard buffer zone is required for persons not involved in handling. The label prohibits the consumption of alcoholic beverages prior to, during, and 24 hours following the application. The label warns that some people may develop a sensitivity to this product. This product can not be used through any type of irrigation system. The labeled reentry interval to treated areas is 72 hours.

Human Illnesses and Injuries

No hydrogen cyanamide-related illnesses or injuries in California have been reported to the Pesticide Illness Surveillance Program of DPR until 1991. A complete illness/injury report for 1992 and 1993 is not available to date.

Dermal Irritation/Sensitization

Hydrogen cyanamide was non-dermal irritant (Toxicity Category IV) and mild eye irritant (Toxicity Category III) in the rabbit (Liggett, 1989; van Beek, 1974). Hydrogen cyanamide caused dermal sensitization in the guinea pigs (Siemer, 1985). Clinical examinations for hypersensitization in workers of a cyanamide production plant indicate that no participants demonstrated hypersensitivity to hydrogen cyanamide during the study (Gloxhuber *et al.*, 1989). However, "it is not possible to generalize from these findings regarding the potency of hydrogen cyanamide without further information on the population from which the study subjects (were) derived" (O'Malley, 1991). Another finding in the hypersensitization study was the history of "cyanamide flush" reactions following alcoholic beverage consumption and hydrogen cyanamide exposure in all of the subjects.

Animal Metabolism

Deitrich *et al*. (1976) were the first to isolate a major metabolite from hydrogen cyanamide in rodents. Using ^{14}C-cyanamide injected intraperitoneally (ip) into rats at $1x10^{-2}$ mg/kg they collected CO_2, urine, and liver tissue to follow the metabolism of hydrogen cyanamide. A single major metabolite was initially isolated by plate and paper electrophoresis. Larger quantities for physicochemical determinations were later isolated using

column chromatography to collect the peak radioactive fraction. Radioactivity for mass balance was determined from aliquots of urine dried in planchets and from CO_2 collected in 1N NaOH from expired air and combusted tissue. These authors found that six hours after treatment about 94 percent of the radioactive label was in the urine and just over one percent was expired as CO_2. At one hour post-treatment the liver contained seven percent of the radioactive label, but this had fallen to zero percent by four hours post-treatment. The proportion of label in metabolite versus that in parent compound was not quantified.

Shirota *et al*. (1984) identified the major metabolite of hydrogen cyanamide in rodents, beagles, and humans as N-acetylcyanamide. Their identification was based on comparing physicochemical properties of synthetic metabolites and their derivatives to the isolated natural metabolite and its derivatives. They also followed the mass balance metabolism of cyanamide in beagles. Dogs were dosed with 1.7 mg/kg ^{14}C-cyanamide by gavage or intraperitoneally. Urine and feces were collected for 27 hours post-treatment. Total recovery in all cases was greater than 80%. The distribution of metabolites at 27 hours was very similar for oral and ip dosing: acetylcyanamide in urine - 86 percent oral and 84 percent ip; cyanamide in urine - 11.6 percent oral and 11 percent ip; radioactivity in feces - 0.3 percent oral and 0.6 percent ip. These results show that most of the oral dose was absorbed in the gastrointestinal tract (oral recoveries were 86-99 percent of the dose), that the metabolism of cyanamide by oral and parenteral introduction was the same, and that elimination occurs rapidly through excretion in the urine. Although the authors identified acetylcyanamide in the urine of human patients being treated with cyanamide they did not follow the kinetics of metabolism in humans.

The registrant has submitted data from both rodent and human experiments performed in their laboratory (SKW Trostberg, 1990). These experiments used high purity (99.5 percent) cyanamide for rodent and human dosing. Male Wistar rats were dosed by gavage at 10 mg/kg and housed individually in metabolism cages. Urine was collected for 48 hours at about 12-hour intervals. Ion chromatography was used to measure acetylcyanamide in urine. On a molar basis an average of 45.6 percent of the cyanamide dose was recovered as acetylcyanamide in urine at 48 hours. Most of this dose had been excreted by 22 hours. No assay was done for untransformed cyanamide.

The experiment in human volunteers provided similar results. Six subjects, each ingested 20 mg of cyanamide in an aqueous solution. Urine samples were collected from these volunteers in 12 hour aliquots for 48 hours. Blood samples were also drawn prior to dosing and two and four hours post-treatment. Urine was analyzed for acetylcyanamide and for thiocyanate by ion chromatography. Blood was analyzed for cyanide measured as hydrogen cyanide by headspace gas chromatography using a nitrogen-phosphorus detector. Blood levels at two and four hours were not significantly different than the pre-treatment controls. There was also no change in the level of thiocyanate in the urine. Hydrogen cyanamide was excreted as acetylcyanamide in human urine. After correction for stoichiometry an average of 40 percent of the oral cyanamide dose was recovered in the urine at 48 hours post-treatment. Ninety-five percent of the recovered dose was eliminated in the first 24 hours post-treatment. This result compares favorably with the result of oral dosing in rats and demonstrates that acetylcyanamide is a good candidate for biological monitoring. Assuming that systemic and oral dosing in man follow the same metabolic path (as demonstrated above in beagles), this 40 percent kinetic recovery can be used to estimate actual human dosage from urine residues.

Dermal Absorption

The same six human volunteers that participated in the metabolism study (see metabolism section) were also used in a dermal absorption study (SKW Trostberg, 1990). Subjects were dosed with a total of 20 mg of

cyanamide applied as a one percent solution (10 mg to each arm). This solution (1 mL) was applied to a 4x4 cm gauze pad which was covered with a rubber pad and affixed to the inner forearm. Exposure was for six hours and then the application site was washed with water and dried. The gauze pads were frozen and later analyzed for cyanamide. Urine samples were again collected for 48 hours at 12 hour intervals. The acetylcyanamide recovered in urine was stoichiometrically converted to cyanamide and this dosage value was used to estimate dermal absorption. To correct for other routes of elimination average dermal absorption was calculated as:

$$\frac{\text{mg cyanamide recovered from 20 mg dermal dose}}{\text{mg cyanamide recovered from 20 mg oral dose}} \times 100 = 2 \text{ percent}$$

This study is not fully adequate to estimate human dermal absorption due to several problems. First, the hydrogen cyanamide was not applied directly to the skin. Analysis of the gauze pads showed that nearly 90 percent of the dose was retained in the gauze. Thus the actual dermal dose is uncertain. Second, an occlusive pad was used to cover the application site. And, third, the exposure time was only six hours. The cumulative effect of these variations from standard protocol has an unpredictable impact on estimated absorption. Consequently, a well conducted animal study may be more appropriate for estimating dermal absorption for exposure assessment.

In a rat dermal absorption study, four male rats (201-233 grams) were used for each sacrifice time, except for control and additional treatments with unlabeled hydrogen cyanamide (LeVan, 1989). All rats were acclimatized and inspected by the performing laboratory. Prior to dose administration, hair on the application site (back and shoulders) was shaved and the shaved area was washed with acetone. The protective appliance (a plastic rectangle; 2.5 cm x 5 cm) was glued to the shaved area with a cyanoacrylate-based glue. Silicone medical adhesive, Type A, was used to seal around the outside of the enclosure. An Elizabethan collar was placed around each animal's neck. A non-occlusive pad (filter paper) was affixed to the plastic enclosure after dose administration.

Doses of hydrogen cyanamide used in this study were 0.1, 1.0, and 10.0 mg per rat equivalent to 8, 80 and 800 ug/cm^2 . Non-labeled (Dormex™ 49% w/w aqueous solution) and ^{14}C-labeled hydrogen cyanamide (radiopurity 87-90 percent with a specific activity of 15 mCi/mmole) were mixed in deionized water. The dosing solution was adjusted to pH 4.5 with phosphoric acid. The pH was adjusted to stabilize the dosing solution and to conform to the pH of the product. One hundred microliters of the dosing solution was administered to the 12.5 cm^2 shaved area with the aid of a glass rod spreader. All treated rats were individually housed in stainless steel metabolism cages which allowed separate collection of urine and feces. Each rat was supplied with water and Certified Rodent Chow #5002 ad libitum.

The exposure times were 0.5, 1, 2, 4, 10, and 24 hours. At the conclusion of the exposure period, rats were anesthetized with halothane and killed by exsanguination. The dosed skin site was excised from the animal. Five percent soap solution (Ivory liquid) and deionized water was used to wash dosed skin and plastic enclosure. A glass rod was used to gently agitate the dosed skin surface during the washing procedure. Samples collected for the analysis were: urine, feces, blood, carcass, dose cell cover, dosed skin and appliance washes, cage wipe, and washed skin. All samples were prepared for analysis by liquid scintillation counter. Results were reported as percent of applied dose.

Penetrated dose of all dose groups increased with increasing dose levels and with increasing exposure times. In contrast, the percent of applied dose in washed skin (bound skin residues) was relatively stable at approximately 7 percent. The majority of the applied dose for all dose groups was recovered as unpenetrated dose (78.3-98.8 percent). Percent applied dose in blood was negligible and was not accounted for in dermal absorption estimates.

Bioavailability of bound skin residues of all dose groups can not be determined because of the short observation times after a ten or 24-hour exposure. The desirable observation time should be up to one week in order to evaluate the bioavailability of bound residues. From the available data, it is appropriate to determine the mean percent dermal absorption by addition of percent penetrated dose and percent dose remaining in washed skin of all dose groups. These dose groups are expected to cover the range of worker exposure to hydrogen cyanamide. The mean percent dermal absorption after correction for total dose recovery was determined to be 11.2 percent.

Dislodgeable Foliar Residue

Hydrogen cyanamide is used on grapes during dormancy when the vines bear no foliage.

Worker Exposure

During December 1987 and January 1988, mixer/loaders and applicators were monitored as they applied hydrogen cyanamide to dormant grapevines in the Coachella Valley (Siemer, 1990a). Workers applied a two or four percent (v/v) solution of Dormex™ using high (300 psi) or low (75 psi) pressure double-row boom sprayers. About 23 percent of the gallons applied were as the two percent solution, which is less than the recommended label rate of four to seven percent. Applications were made using both open and closed cabs. A separate accounting of exposure to applicators working in the two cab types and at the different pressures was done. Monitoring of the mixer/loader task was also separated into chemical loading and water loading. Workers wore Tyvek coveralls over work clothing, nitrile gloves, and half-face respirators. These respirators were equipped with felt dust filters, but the cartridge type used was not specified.

This study used 4 X 4 inch α-cellulose patches for body exposure dosimetry and felt respirator pads to monitor inhalation exposure. It is not clear if the felt pads were backed with an organic vapor cartridge. Eleven patches at appropriate monitoring sites were used. They were pinned and taped to Tyvek coveralls that the workers wore over their standard work clothing. Hydrogen cyanamide residues were extracted from 25 cm^2 circles punched from these patches. Potential dermal exposure was extrapolated from the measured residues using surface areas from Berkow (1931). Two patches under the Tyvek coveralls were used to estimate the percent clothing penetration of Dormex™. Potential inhalation exposure was based on residues extracted from the felt respirator pads (two per worker).

Hand exposure was estimated from hand washes. These were done in plastic bags using a solution of 0.1 percent Tween 20 (v/v) in water. Cyanamide residues were converted to a red complex with penta-cyanoamine-ferrate(II) at pH 10.5 and measured with a Perkin-Elmer UV spectrophotometer. They were corrected for stoichiometry and laboratory spike recovery.

No correction was made for field spike recoveries. The registrant has provided minimal information on the recovery of hydrogen cyanamide residues from field spikes. According to the information provided, recovery

from all field spikes was greater than 50 percent. Individual field spike recovery values should have been submitted. Tank mixes were sampled and analyzed. There was considerable variability in the percent a.i. in these mixes. In some cases less than 50 percent of the intended a.i. was present.

The collection and analysis of the felt pads as a measure of inhalation exposure was questionable. The registrant contended that because of the highly hygroscopic nature of hydrogen cyanamide, the cyanamide particles will always remain associated with the spray droplets and inhalation of the vapors should not be of any concern (Rieder and Mathias, 1990).

Although the chemical and water loading tasks may be done by two workers for applications on large ranches, they are likely to be done by the same worker on small ranches. To estimate exposure to this worker values for the chemical and water loader are combined and presented as a single mixer/loader value. Applicator values for different pressure and cab combinations are presented as a range. Some generalizations about these individual combinations can be made. Applicator exposure during high pressure spraying was higher than during low pressure spraying. Applicator exposure during high pressure spraying was reduced when using a closed cab.

Clothing penetration factors were derived using the interior patch residues. Individual penetration values were found to vary from 0.1 to 99 percent with a geometric mean of 13.4 percent. Body dermal exposure values in Table 1 were estimated from potential exposures and assuming 13.4 percent clothing penetration. Based on this study, inhalation exposure accounted for 10-22 percent of the absorbed dosage in applicators. The lower end of the range of exposure for applicators in Table 1 resulted from the use of low pressure sprayers. The estimates of exposure for a chemical mixer/loader and an applicator wearing all the label-required protective equipment are also shown in Table 1.

Urine samples were also collected from workers during the 1987-1988 dosimetry study described above (Siemer, 1990b). These samples were collected as 24-hour voids following worker exposure to Dormex™. These voids were collected in one gallon bottles from mixer/loaders, applicators, and supervisors. No consistent attempt to refrigerate these samples was made during collection and no preservative was used. The stability of the monitored metabolite under this collection regime was not demonstrated. If workers failed to use the collection bottle for more than two voids during the collection period, their sample was excluded from analysis. Daily (24 hour) urine samples were obtained from workers as they continued to work with hydrogen cyanamide during the application season. One 24-hour void from each worker was also collected more than one month after the end of Dormex™ applications.

A second set of urine samples from mixer/loaders, applicators, and supervisors was collected during the 1988-1989 application season. During this season Dormex™ was applied as a 4-7 percent solution. Additional protective clothing (a chemical resistant apron and boots) was worn by mixer/loaders during this second season. These urine samples were collected in the same way as during 1987-1988, but no passive dosimetry data was collected. There were fewer daily urine samples per worker in this study year. A post-season sample was again collected. Prior to the start of this application season the workers received special use and safety training.

Table 1
Estimates of Hydrogen Cyanamide Daily Exposure and Absorbed Daily Dosage (ADD) for Dormex™ Handlers from Dosimetry

Work Task	Body dermal* exposure mg/8 hours	Hand exposure mg/8 hours	Inhalation exposure mg/8 hours	ADD** ug/kg/day
Chemical+water/mixer/loader***	84-667	0.8-1.8	0.2-0.8	137-1076
Chemical mixer/loader****	234.7	1.6	0.4	15.4
Applicator***	3-41	0.03-0.5	0.2-0.9	6-73
Applicator****	15.9	0.2	0.2	3.0

* - Based on 13.4 percent of potential dermal exposure.
** - ADD is based on a 11.2 percent dermal absorption, 50 percent and 100 percent inhalation retention and absorption (Raabe, 1988), respectively, eight-hour workday, an average 70 kg body weight observed in workers. *** - Clothing consisted of a long-sleeved shirt, long pants, chemical resistant gloves, and boots. **** - Clothing and equipment consisted of a long-sleeved shirt, long pants, chemical resistant suit (95% protection), gloves, boots, and respirator (90% protection); mixer/loaders wearing rubber aprons (90% protection) also (Thongsinthusak et al., 1993).

Brodberg, 1990 and Formoli, 1993, WH&S

Following collection of the 24 hour voids from workers, the volume of each urine sample was measured and brought up to the nearest 25 mL using distilled water. Prior to dilution a 3 mL sample was collected for creatinine determination. Samples were split into 500 mL aliquots and frozen for shipment to Mid State Laboratories or SKW for analysis.

N-acetylcyanamide was the hydrogen cyanamide metabolite measured in urine for biological monitoring. Its stability during collection and storage was not demonstrated by the registrant. Urine was diluted if necessary, adjusted to pH 11-11.5, and purified with charcoal. Acetylcyanamide was quantified as its sodium salt using a Dionex 2010 ion chromatograph. The limit of detection of this method is 0.1 ppm. No correction was made for creatinine content or field spike recoveries. Results were reported as cyanamide after correcting for lab spike recovery and the stoichiometric relationship between cyanamide and acetylcyanamide.

The cyanamide values calculated by the registrant contained a small error in the stoichiometric correction for cyanamide dosage. They used 0.512 instead of the correct value of 0.5 to correct for the difference in molecular weight of cyanamide and acetylcyanamide. Their results are consequently a very slight overestimate of cyanamide levels. Worker Health and Safety Branch has not recalculated these values. The values calculated by the registrant were corrected for the 40 percent recovery of cyanamide as acetylcyanamide in the urine (see metabolism section).

The Absorbed Daily Dosage (ADD) derived from these biomonitoring studies for different types of workers exposed to Dormex™ are shown in Table 2. The registrant did not express this dosage normalized to an eight-hour exposure. The length of workdays in both studies were similar, ranging from several hours to greater than eight hours. Since these represent typical work times for the mixing/loading and application of hydrogen cyanamide, these biomonitoring-derived estimates are meaningful indicators of exposure and dosage. They are within the range of absorbed dosage derived from the dosimetry estimates (compare ADD in Table 1 and 2) considering the fact that biomonitoring values are based on additional protective clothing and equipment and not normalized to an eight-hour workday.

Dosage estimates based on biomonitoring vary over the worker replicates during the season. These data do not show a buildup of excreted dose during the season. Thus the mean in most cases is similar to a single day's dosage estimate.

Table 2
Hydrogen Cyanamide Absorbed Daily Dosage for Dormex™ Handlers Based
on Biological Monitoring of Urine in 1987-1988 and 1988-1989 Seasons

Work Task	Mean (Geometric) ADD* (ug/kg/day)	Number of Workers	Number of Observations
Mixer/loader[a]			
1987-1988 Season	341 ± 3	2	14
1988-1989 Season	6 ± 5**	5	8
Applicator[b]			
1987-1988 Season	12 ± 8	6	42
1988-1989 Season	5 ± 5	10	22
Supervisor[c]			
1987-1988 Season	37 ± 3	2	18
1988-1989 Season	3 ± 9	3	5

* - ADD is based on a 70-kg worker (average body weight of workers in season 1) working for a typical but undefined number of hours in a day.
** - Arithmetic mean (normally distributed)

Protective clothing and equipment (over normal work clothing):
a) In both seasons Tyvek coveralls, half-face respirators, and chemical resistant gloves were worn by mixer/loaders. In the second season these workers also wore chemical resistant aprons and boots.
b) In both seasons applicators wore Tyvek coveralls, half-face respirators, and chemical resistant gloves. In the first season some used high pressure sprayers. It is not clear if any used high pressure sprayers during the second season, but the Section 18 permit conditions in 1988 limited the application equipment to low-pressure closed cab sprayers only.
c) Work clothing worn. In the first season supervisors actively maintained boom sprayers and pumps. Their activity during the second season was undocumented.

Brodberg, 1990 and Formoli, 1993, WH&S

Supervisors during both seasons actively participated in various maintenance and work activities. During 1987-1988 they adjusted pumps and unclogged nozzles. They wore work clothing only, so their dosage level is not unexpected.

There is a trend for all worker dosages derived from biomonitoring to be lower during the 1988-1989 season (see Table 2). This is most striking for the mixer/loaders who show about-60 fold reduction in dose to a level almost equal to that seen in applicators. Since the percent active ingredient mixed during the second season was equal or higher than that in the first season, the reduction is probably due largely to efficient use of the closed loading system, additional protective clothing (chemical resistant apron and boots), and conduct of safety seminars.

A third set of 24-hr urine samples were collected from hydrogen cyanamide handlers during 1992-1993 use season in Coachella Valley, California (Siemer, 1993). The applications (4% Dormex™ v/v) were made under a Section 18 emergency exemption, using low pressure closed cab equipment. Applicators wore rubber suits, rubber gloves, respirator, rubber boots, and goggles. Applicators assisted in mixing/loading, creating potential exposure to the pesticide concentrate. Mixer/loaders wore rubber aprons in addition to the protective clothing and equipment described above. Supervisors wore the same protective clothing and equipment as those of applicators when assisting in handling. The applicators in ranches three and four and a mixer/loader in ranch three wore Tyvek coveralls over their rubber suits.

The study was conducted to meet the requirements of EPA's good laboratory practice standards. Urine samples were collected during exposure to Dormex™. Samples were collected in containers with insulated cooling wraps filled with ice. Collected samples were shipped to the laboratory on dry ice. Urine samples for blanks and spikes were collected from two workers not exposed to hydrogen cyanamide. A sample was not analyzed if the worker skipped two or more voids during the 24-hour period, or the volume was less than 750 mL, or the creatinine level was not within normal range. Samples were analyzed for N-acetylcyanamide. N-acetylcyanamide recoveries from field spiked samples averaged 82 percent. The results were corrected for laboratory recoveries, for the difference in molecular weight of hydrogen cyanamide and acetylcyanamide, and for an eight-hour workday. Table 3 shows ADD values corrected for field recoveries (82%) and for the pharmacokinetic recovery (40%) of hydrogen cyanamide as N-acetylcyanamide in the urine.

Based on the similarity of the biomonitoring and dosimetry estimates for 1987-1988 season (compare ADD in Table 1 and 2), biomonitoring may be the best estimate of worker dosage for risk assessment. The estimates of exposure derived from 1992-1993 (biomonitoring) data are slightly higher than those of 1988-1989 (biomonitoring). This may be because of the inadequate safety training during 1992-1993 season since unsafe work practices were noted in field observations. In addition, the estimates of ADD in the latest season were corrected for field recoveries and for an eight-hour workday. For the same reasons, the ADDs derived from 1992-1993 season may be the most health conservative estimates of handlers' exposure to hydrogen cyanamide.

Hydrogen cyanamide application to grape vines is a seasonal activity that must be made approximately one month before bud break and bud break is dependent on the variety. Growers apply the product themselves, using their own specialized spray rigs such as those used for gibberellic acid applications (Haskell, 1993, Appendix 1). The applications take a few days during the season in small operations. Large operations (up to

Table 3
Hydrogen Cyanamide Absorbed Daily Dosage for Dormex™ Handlers Based on Biological Monitoring of Urine for 1992-1993 Season

Work Task	Mean (Geometric) ADD (ug/kg/day)	Number of Observations
Mixer/loader	6 ± 10	9
Mixer/loader*	16 ± 25	4
Applicator	11 ± 5	21
Applicator*	14 ± 4	11
Applicator**	9 ± 7	10
Supervisor*	2 ± 38	4

Protective clothing and equipment (over normal work clothing):
Rubber suits, rubber gloves, respirator, rubber boots, and goggles; mixer/loaders wore rubber aprons also; some workers (except supervisors) wore Tyvek coveralls over their rubber suits.
* - Excludes the worker wearing Tyvek over rubber suits. A water loader, and a mechanic/loader were also excluded.
** - Applicators wearing Tyvek coveralls over their rubber suits.

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1500 acres) may spend up to 30 days working four to ten hours a day during the season. An application crew treating 20 acres a day will spend 17 days during the season to treat an average size ranch (340 acres). Table 4 shows the ADD values for 1987-88, 1988-89, and 1992-93 studies, and the weighted average for all three studies. Seasonal average daily dosages (SADD) are calculated based on 12 and 26 workdays of exposure in a two-month period for mixer/loaders and applicators, respectively. The SADD for 30 days of exposure in a season are also shown in Table 4. The average annual daily dosages (AADD) are normalized to a year.

A drift study was conducted at three locations in Coachella Valley, California to provide evidence for discontinuation of the 100-yard (300 feet) buffer zone requirement during use of Dormex™ in California under the Section 18 Emergency Exemption (Hicks *et al.*, 1992). The wind was calm at all three locations with occasional gusting up to eight mph, changing directions from time to time.

Workers applied three to four percent (v/v) Dormex™ spray to grape vines using spray rigs . Downwind distances from the tractor's first spray path were marked approximately 300 feet at each location. The two laterals and upwind sides were marked at 25 and 50 foot distances at each location. Residue sampling media consisted of alpha cellulose pads with 24.6 cm^2 exposed surface were placed at each distance 3.5 feet above the ground on wooden stakes facing the spray area. Staplex high volume air samplers fitted with a TFA41 paper filters (62.1 cm^2 exposed surface) were installed at the height of approximately 3.5 feet to collect air samples downwind. The air samplers were adjusted to draw 20 cubic feet of air per minute.

Table 4
Hydrogen Cyanamide Estimated ADD and SADD
in ug/kg/day for Workers Involved in Handling Dormex™

Work Task	ADD (n) 87-88	88-89	92-93	Average*	Seasonal Workdays	SADD (ug/kg/day)	AADD (ug/kg/day)
Mixer/loader	15 (7)	6 (8)	16 (4)	11.6 ±4.7 (19)	12**	2.3	0.38
					30	5.8	0.95
Applicator	3 (39)	5 (22)	14 (11)	5.3 ±3.8 (72)	26**	2.3	0.38
					30	2.6	0.44
Supervisor		3 (5)	2 (4)	2.6 ±0.5 (9)	30	1.3	0.21

(n) - Number of observations.
* - Weighted average of three studies.
** - Seasonal workday limit required by the U.S. EPA.

Protective clothing and equipment:
Rubber suits, rubber gloves, respirator, rubber boots, and goggles; mixer/loaders wore rubber aprons also.
Using closed system mixing/loading, and closed cab and low pressure sprayers.
Supervisors wore the same protective clothing when involved in handling.

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There were five replicates (pads) at each downwind distance, three replicates at each lateral distance, and five replicates at upwind distances. Two to three air samplers were placed at each downwind distance. Each set of samples was collected after two complete passes of spraying (approximately five minutes, except for air samples).

Alpha cellulose pads and paper filters spiked in the laboratory were shipped on blue ice to the test site. The spiked samples were exposed to the ambient temperature during the study and then processed in the same manner as the field samples for delivery back to the laboratory. All samples were air-transported to the lab on dry ice. Field spike recoveries averaged 77 ± 11 percent. Results were corrected for recoveries.

The efficiency of the paper filters used to measure inhalation exposure is questionable as discussed earlier in the Worker Exposure section. Minimum data were collected for drift to the lateral distances because the droplets from the sprayer were falling directly on the collected sampling media. This was due to the maneuvering of the application equipment at the end of the field while the sprayers were still on. The residues on the collected pads ranged from 14 to 684 ug/cm^2/minute at 25 feet lateral distance and 19 to 221 ug/cm^2/minute at 50 feet lateral distance. Upwind three-location average residues on the pads were 5.36 ug/cm^2/hour and 5.48 ug/cm^2/hour at 33 and 57 feet distances, respectively.

The residue data from the lateral distances are inconclusive because of the direct falling of droplets from the spray equipment on to the sampling media and because no samples were collected at distances beyond 50 feet. The highest upwind drift residue observed at one location (first location), may be due to the shifting wind. As a

result, the average drift residues at upwind were slightly higher compared to that of the downwind at 25 and 50 feet distances. No upwind drift samples were collected at distances beyond approximately 50 feet. Downwind three-location average residues on the pads and air sampler filters declined with the distance from the application site (Table 5).

The observed residue data (ug/cm^2/hr) presented in Table 5 were extrapolated from residues obtained from five minutes of exposure. This will substantially enlarge any error in the assumption that the residue level increases proportionally with an increase in exposure time. The predicted residue data were obtained from log regression correlation analysis of residues observed at various distances. The ADDs were estimated for a bystander based on predicted dermal (pad residues) and inhalation (air residues) exposures during hydrogen cyanamide applications.

Table 5
Hydrogen Cyanamide Downwind Drift Residue
and Estimate of Exposure (ADD) for a Bystander

Distance feet	Pad Residues Observed ug/cm^2/hr	Air Residues* Observed ug/M^3	Pad Residues Predicted ug/cm^2/hr	Air Residues Predicted ug/M^3	ADD** Bystander ug/kg/1-hour day
26	3.54				
47		119.88			
51	3.40				
94		73.22			
100			1.24	60.39	7.5
113	0.94				
140		55.22			
187		22.24			
200			0.68	29.04	4.1
207	0.52				
234		26.44			
277	0.64				
279		20.13			
300			0.48	18.93	2.9

*- Calculated from filter size of 62.1 cm^2 and air sampling rate of 20 liters (0.566 m^3) per minute.

** - One hour/day of exposure for a bystander wearing work clothing (exposed head, face, and hands). Body surface area (19400 cm^2), weight (75.9 kg), and breathing rate during light activity (14 L/minute) according to the pesticide exposure assessment guidelines (Thongsinthusak, *et al.*, 1993). Clothing penetration of 10 percent. Dermal absorption rate of 11.2 percent (see dermal absorption section).

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APPENDIX 1

State of California

Memorandum

To : John Ross, Senior Toxicologist
Worker Health and Safety Branch

Date : August, 19, 1993

Place : Sacramento

Phone: 654-1155

From : **Department of Pesticide Regulation** David Haskell, Associate Environmental -Research Scientist
Worker Health and Safety Branch

Subject : Potential Use Pattern From Proposed Registration of Dormex® on Grapes

The manufacturer of Dormex®, SKW Trostberg Aktiengesellschaft of Trostberg, Germany, has requested a section three registration for the use of this product on grapes to promote earlier and uniform bud break. Dormex® is formulated with water as a emulsifiable concentrate with 51% hydrogen cyanamide by weight. With a specific gravity of 1.06, each gallon contains approximately 4 lbs of active ingredient. The product is applied in the winter months after the vines are pruned and a minimum of 30 days exists before the start of bud break. The use of Dormex® has been permitted through the EPA issuance of a section 18 emergency exemption for the 88-89, 89-90, and 92-93 growing seasons. One application per growing season was permitted at a rate of 50-100 gallons of a 2-4% of Dormex® mixture per acre (4-16 lbs a.i./acre). Applications were made with ground spray rigs equipped with enclosed cabs and closed mixing systems. The season of use permitted by the exemptions ranged from mid-November to mid-February.

The use of Dormex® on table grapes has been limited to the desert counties of Riverside and San Bernardino, primarily in the Coachella Valley. Imperial County requested to be added to the section 18 exemption during one season but no use was reported. Riverside County reported 46 use permits were issued and 15,018 acres were treated during the winter of 88-89. In the winter of 89-90, 40 use permits were issued and 14,306 acres were treated. In December of 1992, the section 18 exemption was issued and then withdrawn on January 15, 1993. Preliminary reports indicate some 35 permits were issued with 11,770 acres treated. One use permit was issued in San Bernardino County in 1988 and 365 acres were treated. The average application rate for the two years of reported usage was 15.3 lbs of a.i. per acre.

The staff of the Riverside County Agricultural Commissioner indicated that one permit is usually issued for each table grape grower (Gillis, 1993). The size of the operations range from 20 acres up to 1500 acres with a mean size of 342 acres. Almost all Dormex® applications were made by the growers themselves using specialized spray rigs for applying gibberellic acid (Synder, 1993). The application work was not contracted out to PCOs because the growers already own the specialized equipment needed for application. The cost of custom application work and the need to have flexiable spray schedules in order to follow

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the grape pruners were other reasons for not hiring PCOs (Sun World, 1993).

Discussions with two large table grape growers provided information on how the applications of Dormex® were actually made (Richard Bagdaseria Co., 1993; Sun World, 1993). The applications are timed to follow pruning as soon as possible and coordinated as to the type and location of each grape variety to meet the 30 day before bud break requirement. Bud break usually starts about February 1st in the frost-free areas and in colder areas about Mid-March (Gillis, 1993). The length of the application season is about two months, starting in about early December and finishing in early February.

Applications are made with tractors equipped with folding booms that can spray both sides of two grape rows in one pass. A 500 gallon mix tank is pulled behind the tractor. One load can treat 4-10 acres depending on the dilution rate. Small operations with only 20-40 acres can complete the treatment in a few days with the grower performing all the work tasks (Sun World, 1993). Larger operations may consist of several hundred acres of grapes that are located on ranches in different climatic zones. Spray crews operating several tractors at one time are required to spray each ranch quick enough in order to follow the pruners and keep the ranch synchronized with the other cultural practices that will follow. The work tasks are designated with drivers operating the tractors exclusively and other workers handling all the mixing/loading tasks. All mixing/loading may take place at one central location on the ranch or a nurse rig may bring water to the mixer/loaders as they follow the spray tractors. A spray crew operating 3-4 tractors with one mixer/loader and a nurse truck driver if necessary, can treat an 80 acre vineyard in one day with 80 gallons of mix per acre. Treatments with higher dilution rates (100-125 gallons/acre) and ranches located on rocky ground will take longer to complete. One operation treated 40 acres in four hours operating seven tractors but averaged 50 acres per day for the season. Some of the spray operators for these larger companies may treat 30 days a season with Sundays off, working 4-10 hours per day.

The use of Dormex® on table grapes to promote earlier and uniform bud break is expected to be limited to grapes grown in the desert areas of the state (Sun World, 1993). Acreages located in the southern San Joaquin Valley normally receive enough winter chilling to promote uniform bud break. Use of the product here will incur the risk of bud break taking place during the frost season.

Attachment:

cc: Tareq Formoli

REFERENCES

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Richard Bagdaseria Co., 1993. Personal conversation with production manager on August 16, 1993. Grower has 1500 acres.

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Sun World, 1993. Personal conversation with production manager on August 16, 1993. Company grows on 700 acres with other growers for 700 acres.